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EDITOR
HENRY CHANDLER COWLES

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WITH TWENTY-SEVEN PLATES AND ONE HUNDRED TWENTY-SEVEN FIGURES



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P. 557, line 1 of figure legend, for "Procentrum" read "Prorocentrum"



THE
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February 1929

ROOT FORMATION AND FLOWERING OF DAHLIA
CUTTINGS WHEN SUBJECTED TO
DIFFERENT DAY LENGTHS¹

P. W. ZIMMERMAN AND A. E. HITCHCOCK
(WITH SIX FIGURES)

Introduction

Dahlias propagated from cuttings during late summer and fall vary in response as the days become shorter. Cuttings taken in August produce normal fibrous roots and become plants of good size before flowering. Cuttings taken in late September and October produce storage roots at the sacrifice of fibrous roots, and flower when only a few inches high. Often no roots at all are formed on cuttings taken in October, but the basal ends of the stems serve as storage centers. Occasionally the buds along the stem become storage organs, swelling and resembling tubers. When six hours of extra light are used to supplement the daylight, the plants produce normal fibrous roots and are slow to flower. GARNER and ALLARD (5), ARTHUR and GUTHRIE (1), and ARTHUR (2) have reported many plant species which flower more readily in short day lengths than in long days. GARNER and ALLARD reported one variety of dahlia, John Ehlich, which flowered on July 8 in the 10-hour day as compared with September 27 for the check grown in the natural day length. They also reported that storage roots and tuber formation

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

of several species of plants are affected by day length. Two unnamed dahlia varieties did not show the usual "tuber" formation when grown in lengthened illumination periods. A bean (*Phaseolus multiflora* Willd.) and a yam (*Dioscorea alata*) formed larger storage roots in 10- to 12-hour days than in the full day length of summer. McCormick potatoes did not tuberize with extra illumination added to the usual day length of summer. Artichokes (*Helianthus tuberosus*) in short days formed mature looking tubers in contrast with the slender, immature types of the long day plants.

The purpose of this paper is to report the response of cuttings from several varieties of dahlia when subjected to different day lengths.

Methods and materials

During the late summer and fall of 1926, cuttings were taken at one-week intervals from dahlias growing on the Institute grounds and were planted in the greenhouse in sand or mixtures of peat moss and sand. The material was generally young shoots coming from the main stem rather than from the ends of large active branches. In this way it was possible to get material free from flower buds. After cuttings were rooted, they were potted in good garden soil and grown either in a greenhouse receiving normal fall light or in an adjoining greenhouse where they were given extra light. The extra light was given from sunset to 12 o'clock midnight by a 1500-watt nitrogen bulb.

Seedlings which were also included in the experiment were from a promiscuous collection of seeds planted on November 3. About 1000 seedlings resulted, and from these the best plants were selected for experimental work.

During 1928 the plants used came, for the most part, from select material purchased from Downs Dahlia Farm, Clayton, New Jersey, and from Fisher and Masson, Trenton, New Jersey. The storage roots were placed in a peat moss medium, and cuttings were made as the shoots appeared above the surface. These cuttings were placed directly into pots containing a mixture of peat moss and garden loam. Later the established plants were repotted in fertilized soil and subjected to different amounts of light. One lot was grown outdoors as a check; a second lot by the check was given 7 hours of

daylight and the remainder of the day in the dark room; a third lot by the check was given 9 hours of daylight and the remainder of the day in the dark room.

Nitrate tests were made with a solution of diphenylamin in sulphuric acid (0.1 gm. of diphenylamin to 10 cc. of 75 per cent H_2SO_4).



FIG. 1.—*Dahlia variabilis* (Jersey's Beauty) from cuttings, August 26 to November 15

Hydrogen-ion measurements were made with a quinhydrone apparatus. Stems and leaves were cut into small pieces and then crushed in a mortar. Some of the crushed plant material, as well as the juice, was transferred to the electrode chamber. It will be noted that the sample thus measured contained both tissue and its expressed juice.

Results and discussion

EXPERIMENTS WITH FALL CUTTINGS.—Cuttings taken on August 26 from dahlia varieties, such as Jersey's Beauty, Mrs. I. de ver Warner, or Peg-O-My-Heart, formed normal fibrous roots by Sep-



FIG. 2.—*Dahlia variabilis* (Jersey's Beauty) cuttings, October to December, as they appeared when taken from rooting medium; note almost complete absence of fibrous roots; upper figures, main roots developed are storage organs; lower figures, left, storage in base of new shoot arising after cuttings were made; middle, basal buds form storage organs comparable with tubers; right, portions of cutting, as stem and leaf petiole, serve as storage organs.

tember 17 and many flowered by November 15. They did not flower uniformly, but continued up to December 15. At the time of flowering the plants varied from 1 to 3 feet in height (fig. 1). Five plants given 6 hours of extra light had not flowered by February 7, and neither had they formed storage organs. Fibrous roots and large tops

developed at the expense of storage roots. The extra light plants ranged from 3 to 4 feet in height, while checks varied from 1 to 3 feet in height.

Cuttings taken from the same varieties on September 26 produced a few fibrous roots, but there was a tendency to produce storage roots while still in the rooting medium. Flower buds were formed when the plants were 10-12 inches high. Flowering and the formation of storage roots took place concurrently from October to December.

Cuttings taken from October 15 to October 28 developed storage roots, as a rule, instead of the normal fibrous roots characteristic for early cuttings. In many instances no roots formed, but various parts of the stems became storage organs (fig. 2). In some cases buds developed into organs resembling bulbs or tubers; in others the stems became storage centers; and in still others remaining portions of leaf petioles were enlarged. Where roots formed, as a rule they were few and mostly of the storage type. A few cuttings without roots flowered while in the rooting medium.

EXPERIMENTS WITH SEEDLINGS.—Approximately 100 seedlings grown from seeds planted November 3 were treated with normal and extra light. Much variation was evident, but the prevailing tendency was for the plants to flower and form storage roots in the short winter days. Plants given extra light formed fibrous roots only, and did not flower during the course of the experiment (fig. 3). Seedlings first developed a fibrous root system from the hypocotyl, and when the plants were 4 or 5 inches high, special roots appeared just below the cotyledons. These roots had a fleshy appearance and quickly developed into storage organs during the short days.

EXPERIMENTS WITH SPRING AND SUMMER CUTTINGS.—On June 8, thirty Jersey's Beauty dahlia plants, half from cuttings and half with tubers attached, were divided into three lots of ten each, and given the following treatment:

Lot 1, grown outdoors in normal day length.

Lot 2, grown outdoors for 9 hours each day and the remainder of the time in the dark room.

Lot 3, grown outdoors for 7 hours each day and the remainder of the time in the dark room.



FIG. 3.—Seedling dahlias: *A*, grown from November 3 to February 7; left, plants receiving normal light during winter months flowered when only a few inches high; right, same as those on left in the beginning, but given extra illumination from sundown to midnight: *B*, grown from November 3 to January 4; two large plants with fibrous roots given extra light from sundown to midnight; small dahlias with storage roots received usual light of November and December: note that extra light most completely eliminated formation of storage roots, while short day plants, although only a few inches high, produced unusually large storage roots.

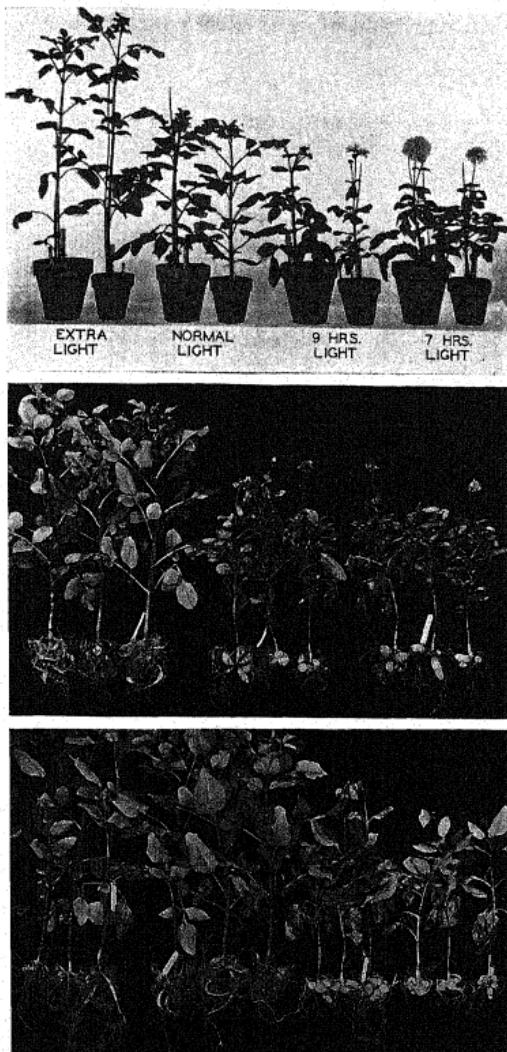


FIG. 4.—Top, *Dahlia variabilis* (Jersey's Beauty), June 8 to July 17, showing effect of length of day on top growth and flowering; plants in large pots had old storage root attached; plants in small pots were grown from cuttings; first flower buds occurred in short day plants on June 26, and on long day plants August 6; all short day plants had flowered by July 17; normal light plants started to flower August 20. Middle, root systems of plants from lots photographed in top row; extra light plants were omitted, otherwise the order is as appears in top row; photographed August 6. Note heavy root storage in short day plants; normal light plants developed fibrous roots with only a slight tendency to produce storage roots; when storage roots started, they were elongated and unlike the storage roots of short day plants. Below, Mrs. I. de ver Warner dahlias showing effect of length of day on roots and tops; labeled as in top row, started June 8 and photographed August 6; this variety responds almost exactly like Jersey's Beauty; in extra light plants where there is a tendency to form "tubers" there is great contrast to the short ball-like storage roots of short day plants.

Table I shows the effect of length of day on the type of roots produced, time of flowering, and the supply of nitrates present. Lots 2 and 3 were very much alike throughout, varying only in size. The normal day plants produced many more leaves and became larger in general than short day plants. Microchemical tests showed a very high supply of nitrates in the leaves of short day plants, while very little appeared in the normal day plants. The hydrogen-ion concentration of expressed sap from stems or leaves deviated only slightly

TABLE I

EFFECT OF LENGTH OF DAY ON TYPE OF ROOTS AND TIME OF FLOWERING OF JERSEY'S BEAUTY DAHLIA; DURATION OF EXPERIMENT JUNE 8 TO AUGUST 6, 1928

| LIGHT TREATMENT | NO. OF PLANTS USED | | DATE OF FIRST VISIBLE FLOWER BUDS | NO. OF PLANTS FLOW- ERING ON JULY 17 | AVERAGE HEIGHT | | TYPE OF ROOTS FORMED | NITRATES (MICRO- CHEMICAL TESTS) |
|-----------------------------|-----------------------|------------------------------|---|--|-------------------|------------------------------|--|---|
| | Cut- tings | With old "tu- bers" | | | Cut- tings | With old "tu- bers" | | |
| Lot 1, normal day length. | 5 | 5 | August 6 | 0* | 2'6" | 3' | Fibrous roots with slight tendency to form storage roots | Nitrates low in leaves and stems |
| Lot 2, 9 hours of daylight. | 5 | 5 | June 28 | 10 | 1'6" | 1'8" | Storage roots with comparatively few fibrous roots | Nitrates abundant in leaves and stems |
| Lot 3, 7 hours of daylight. | 5 | 5 | June 26 | 10 | 1'4" | 1'6" | Storage roots with comparatively few fibrous roots | Nitrates abundant in leaves and stems |

* A few plants remaining after the experiment was discontinued showed flower buds on August 31.

from pH 5.4 in plants of all lots. The normal light plants developed mostly fibrous roots with only an indication of storage, while both the 7-hour and 9-hour plants had large storage roots. Where storage roots had started on normal day plants they were slender, with long necks, whereas those on short day plants were short and rounded like potato tubers (fig. 4).

In order to find whether the presence of stored food material influenced the type of root system or the time of flowering, five plants in each lot were grown with old "tubers" attached. The response was essentially like that of cuttings (fig. 5).

In addition to Jersey's Beauty, six other named varieties have been used in experiments. Of these, four varieties (Mrs. I. de ver

Warner, Trentonian, F.T.D., and Insulinda) formed storage roots and flowered only when given short day treatment. Table I, showing data for Jersey's Beauty, could almost exactly be duplicated for Mrs. I. de ver Warner. The main difference was a slightly earlier bud development in Warner plants. Arthur (pompon), Esther R. Holmes, Maid of Watts, and Summer Red were indifferent to length of day in respect to flowering, but storage roots were favored by short days (fig. 6).

There are many varieties of dahlia that flower throughout the long days of summer. The pompon types, particularly, are known for this characteristic. Fig. 6 shows two pompons (Arthur) which flowered in both long and short days. They show, however, a great difference in root storage. The photograph showing roots was taken on August 8, after the days were beginning to shorten. Note that the long day plant has many fibrous roots, but carbohydrate storage had started. The short day plant, however, has much heavier storage. In this case it is clear that carbohydrate accumulation is independent of flowering, but is controlled by the length of day. Also flowering is independent of storage root formation, for in the long day plant, flowering had been accomplished before storage had started in the roots. In types like Jersey's Beauty, flowering and the development of storage roots took place concurrently in the short days. Long day plants could form neither storage organs nor flowers (fig. 5). It so happens that the same factor, the length of day, controls both responses at the same time in this type of dahlia, although root storage and flowering are probably not correlated in a causal way. This supposition is strengthened by the fact that plants grown with old storage roots attached responded to day length almost exactly as did cuttings (fig. 6). "Tubers" attached to the pompon dahlias did not change their flowering response, as these plants flowered under all conditions.

Temperature was not controlled in any of the experiments with dahlias. Possibly both light and temperature could be determining factors. In the case of potatoes, GARNER and ALLARD (5) showed that tuberization could be completely eliminated by extra illumination. BUSHNELL (3), SMITH (7), FITCH (4), and others completely eliminated tuberization of potatoes by keeping the temperature up

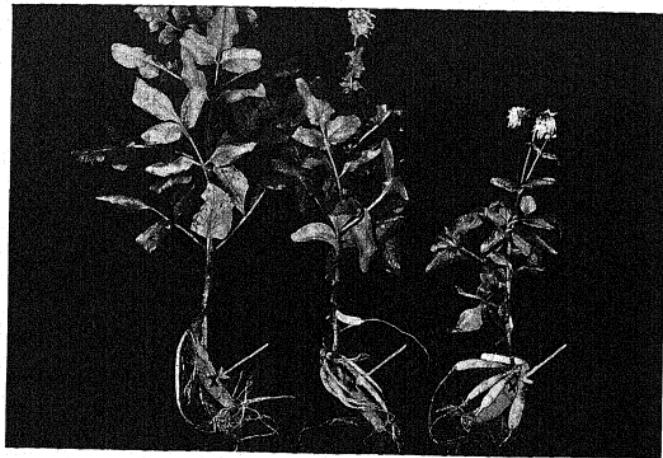


FIG. 5.—*Dahlia variabilis* (Jersey's Beauty) plants with old storage root attached, June 8 to August 6; left to right, normal light, 9 hours' light, and 7 hours' light: note that short day plants flower and produce storage roots the same as do plants grown from cuttings; there is no noticeable influence from the old storage root (indicated by black cross).

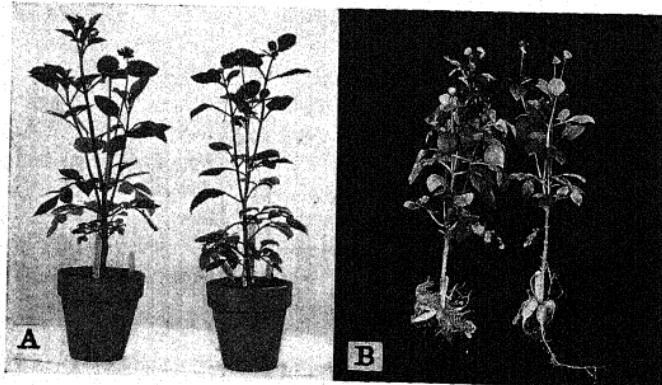


FIG. 6.—*Dahlia variabilis* [Arthur (pompon)]: A, plants from cuttings, June 8 to July 16; plant on left received normal light; plant on right received 9 hours of light; this variety flowers on both long and short day illumination; B, plants same as those photographed in A, but grown from June 8 to August 6: note that root types are same as in other varieties, although flowering is irrespective of light.

to 29° C., while plants at 20° C. tuberized heavily. This suggests that work ought to be done in which both light and temperature are taken into consideration.

The effect of light on cuttings while they are in the rooting medium has long been a subject under discussion. The point of interest has usually been the effect of light intensity. In case of dahlia both light intensity and duration are important. Vegetative growth is so limited by short light duration that few or no fibrous roots can be produced. Such roots as can grow are storage in nature. Many variations from normal storage roots have been noted. Old stems become modified at points where carbohydrates accumulate, and the lowest buds develop into tuber-like organs (fig. 2). Leaves placed in the rooting medium often formed roots, but whether they rooted or not, the basal end of the petiole became enlarged and took the place of storage tissue. Cuttings grown earlier in the season, when the days were longer, had no such tendency toward developing storage organs. Normal fibrous roots were the rule. There is doubtless a certain day length where both vegetative growth and storage can proceed at an even rate. On either side of this particular duration one would have an advantage over the other.

It is of interest to note that nitrates accumulate in the short day plants (table I). NIGHTINGALE (6) found that *Salvia*, buckwheat, and soy beans illuminated for only 7 hours each day had a higher percentage of nitrates and carbohydrates than the long day plants. He states that carbohydrates accumulate in the short day plants, presumably because there is relatively little utilization of them in the synthesis of nitrates to other forms of nitrogen. Even though growth is checked, if the plant remains in good condition, carbohydrates will continue to be manufactured and nitrates to be absorbed. It seems reasonable to suppose that when any growth process is interfered with, the utilization of the two main building materials, carbohydrates and nitrates, will correspondingly decrease, thus permitting their accumulation. In the case of the dahlia, a 7- or 9-hour day quickly brings vegetative growth to an end and flowering is accomplished. The plant remains in good condition thereafter for a considerable period of time, continuing to manufacture carbohydrates and absorb nitrates. As these two substances accumulate, the plants take on the appearance of approaching dormancy. They

do pass, in time, into complete dormancy and lie for two or more months before they are able to grow. If they are changed early enough from short to long day, dormancy is prevented and vegetative growth starts.

Summary

1. Length of day determines the type of root system formed by cuttings for 6 varieties of dahlia, heavy root storage being correlated with a short day, and a fibrous root system being correlated with a long day.
2. Flowering was found to be independent of storage root formation, although certain varieties, such as Jersey's Beauty and Mrs. I. de ver Warner, flowered and formed storage roots concurrently on a short day.
3. Certain varieties, such as Arthur and Summer Red, flowered independently of day length; while others, such as Jersey's Beauty and Warner, flowered only on a short day.
4. Cuttings taken during late September and October developed various types of storage organs along the stem without forming either typical storage roots or fibrous roots.
5. Nitrates accumulated in the leaves and stems of short day plants, but were absent or present in only small amounts in long day plants.
6. The hydrogen-ion concentration of stems or leaves of long and short day plants varied only slightly from pH 5.4.

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YONKERS, N.Y.

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MOSAIC DISEASE OF TOBACCO¹

C. G. VINSON AND A. W. PETRE

Introduction

BEIJERINCK (3) reported working with an infectious juice expressed from plants with mosaic disease. He obtained a precipitate with alcohol from the juice. This precipitate, after drying at 40° C., retained its infectivity. HEINTZEL (8) obtained the same results as BEIJERINCK. ALLARD (1), VINSON (16), and SMITH (15) have also reported the use of alcohol in precipitating the virus from juice of diseased plants. ALLARD (1) has shown that an active fraction can be adsorbed from juice of diseased plants by talc and aluminium hydroxide. He also obtained a glycerin extract from dried, ground leaf material which, after filtering, appeared to contain more of the original activity than the residue. VINSON (16), in addition to corroborating the observations on precipitation by alcohol, reported analogous behavior with acetone, ammonium sulphate, and safranin. BREWER, KRAYBILL, and GARDNER (4) reported activity in a colorless solution obtained by adsorbing the virus on charcoal, washing out the coloring matter, and then freeing the virus.

The biological reaction is the only test available, so far as known, for determining the presence of the virus. No distinction can be made as to the relative virus concentrations when the methods of inoculation in general use are employed. MCKINNEY (12) has called attention to the desirability of a method for determining virus concentrations, and has devised a method which detects considerable differences in virus concentration. The method of HOLMES (9), based on the introduction of a small dose, detects differences in virus concentration within more restricted limits.

This paper gives in detail the results obtained in removing the virus from infectious juice by methods previously described (16), and also reports further progress in freeing the virus of accompany-

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

ing solids. We submit evidence, based on measurements obtained by an adaptation of HOLMES' technique, that the virus of mosaic disease of tobacco can be freed of 90 per cent of the non-active solids of the juice with no apparent loss in infective power.

Preliminary work

Only diseased tomato plants were at first available. Leaves and tips of these plants were collected from the field. Part of this material was treated with ether (5) in the fresh condition, and the cell sap expressed at once with a hydraulic press. The other part of the plant material collected was placed in a cold room and allowed to freeze. It was then thawed and the cell sap expressed. Both lots of juice proved to be infectious.

On concentration in *vacuo* the juice frothed badly, especially that from the etherized plants. Caprylic alcohol and diphenyl ether were successfully used as foam breakers. A few drops of either of these substances reduced the tendency to froth considerably, but after their use the juice became inactive, even though the temperature of the water bath was never allowed to rise above 50° C. It was found, however, that juice from frozen plants could be concentrated in *vacuo* without troublesome frothing, the flask requiring close attention only when boiling began.

An attempt was made to remove some of the solutes present in the concentrated juice by electrodialysis. The first solution electrodialyzed was one prepared by concentrating 2300 cc. of juice from frozen tomato plants to 150 cc. To the 150 cc. of concentrate 20 per cent, by volume, of ethyl alcohol was added as a preservative. This solution was infectious. Its pH was 4.76. In the electrodialysis work a Y tube was used, and the ends of the two arms covered with linen cloth impregnated with collodion. The arms on the Y were inserted into two separate small beakers of distilled water containing a trace of magnesium sulphate as electrolyte. Platinum electrodes were inserted in the beakers, consequently the electrodes did not come in contact with the concentrated juice. The Y tube was then filled up to the stem with the concentrated juice. The dialysis was allowed to run for about 18 hours, the current being about 10 milliamperes at 20 volts. When the experiment was terminated, samples were

taken from both of the beakers and both arms of the Y tube. That from the arm of the tube projecting into the beaker which contained the negative electrode was infectious. The other samples were not infectious. The experiment was repeated on a second portion of the same sample, and the same results were obtained. This indicated that the active principle, under certain conditions, carried an electric charge.

Experimental work

PREPARATION OF MATERIAL

Tobacco plants were used in the following experiments. The original stock of virus was obtained from Professor JAMES JOHNSON,

TABLE I
DATA SHOWING MOST OF VIRUS IS OBTAINED IN JUICE EXPRESSED FROM
TISSUES FIRST FROZEN, THEN THAWED

| DILUTION | EXPRESSED JUICE 700 CC. | | FIRST AQUEOUS EXTRACT OF PRESS CAKE, 600 CC. | | SECOND AQUEOUS EXTRACT OF PRESS CAKE, 604 CC. | | THIRD AQUEOUS EXTRACT OF PRESS CAKE, 523 CC. | |
|----------------|-------------------------------------|-----------------|--|-----------------|---|-----------------|--|-----------------|
| | No. of plants inocu- lated | No. diseased | No. of plants inocu- lated | No. diseased | No. of plants inocu- lated | No. diseased | No. of plants inocu- lated | No. diseased |
| Undiluted..... | 10 | 10 | 10 | 8 | 10 | 7 | 10 | 6 |
| 1/250..... | 10 | 6 | | | | | 10 | 0 |
| 1/500..... | 10 | 6 | 10 | 1 | 10 | 1 | | |
| 1/1000..... | 10 | 6 | 10 | 2 | | | | |
| 1/5000..... | 10 | 4 | | | | | | |
| 1/10000..... | 10 | 2 | | | | | | |

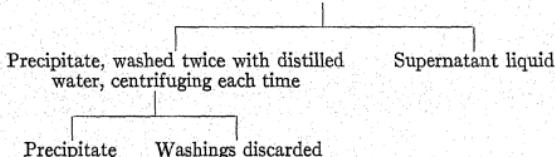
Department of Plant Pathology, University of Wisconsin. This virus gave the "marked mottling, malformation and stunting" characteristic of the mosaic disease of tobacco as found in the field. The juice samples on which our observations have been made were obtained from frozen diseased leaves, or from leaves and stalks after thawing at room temperature. The juice was pressed out of the thawed material (without grinding) at 500 pounds per square inch, then centrifuged for 10 minutes at 1500 r.p.m. This gives, in the centrifuge used, a centrifugal force about 600 times that of gravity. The juice obtained is brown, and free from large suspended particles. That obtained only from leaves contains about 22 per cent of the leaf solids and 12.5 per cent of the leaf nitrogen. That the juice obtained

by this method from diseased plants contains the greater portion of the virus is shown by the infectivity of the expressed juice, as compared with the aqueous extracts of the ground press cake. Results of tests of juice and extracts are given in table I.

PRECIPITATION OF VIRUS WITH AN AQUEOUS SOLUTION OF SAFRANIN

The results in electrodialysis, and the fact that ALLARD (1) was able to remove the virus from solution with talc, gave evidence that the virus possessed some properties characteristic of colloids. It was natural, therefore, to expect that certain substances which could neutralize the charge or affect the water relationship of the active principle might throw it out of solution. Solutions of certain dyes were therefore added to samples of juice from diseased tobacco plants. Salts of acid dyes such as crystal ponceau and rose bengal gave a precipitate when added to juice from diseased plants. That produced by rose bengal settled rapidly, but when the supernatant liquid was tested it was found to be active. Solutions of basic dyes such as Bismarck brown and safranin were tried. Bismarck brown gave a precipitate which settled rapidly, but the supernatant liquid retained activity. Ten cc. of a 1 per cent safranin solution when added to 50 cc. of juice from diseased plants gave a precipitate which flocculated and settled very slowly. The supernatant liquid was, according to our determinations, almost free from virus. Safranin, therefore, was the only one of these dyes found capable of precipitating the virus almost completely. It is to be expected, however, that other azine dyes will also precipitate the virus.

The safranin precipitate was obtained according to the following scheme. Juice from diseased plants was treated with a 1 per cent aqueous solution of safranin in the proportion 10 to 50 cc. of the juice, then placed in the ice box for several hours, usually overnight, and centrifuged:



FREEING VIRUS FROM SAFRANIN PRECIPITATE

Safranin being a weak base, it was thought that by adding a small amount of acid or a small amount of stronger base to a suspension of the safranin precipitate, the virus might be set free. Table II gives the results obtained in preliminary attempts to free the virus from the precipitate by acid and alkali treatments. As indi-

TABLE II
ATTEMPTS TO FREE VIRUS FROM SAFRANIN PRECIPITATE BY ACID AND
ALKALI TREATMENT

| EX- PERI- MENT NO.* | PREPARATION | TREATMENT | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|------------------------------|--|---|-------------------------------------|-----------------|
| 1.. | (a) Precipitate suspended in 0.5 per cent agar solution | None | 10 | 1 |
| | (b) 15 cc. of suspended precipitate | +3 cc. N/14 HCl | 5 | 1 |
| | (c) 15 cc. of suspended precipitate | +3 cc. N/14 NaOH | 5 | 0 |
| | (d) 30 cc. of supernatant liquid | +3 cc. N/14 HCl | 5 | 0 |
| | (e) 30 cc. of supernatant liquid | +3 cc. N/14 NaOH | 5 | 0 |
| 2.. | (a) Precipitate suspended in 0.5 per cent agar solution | None | 10 | 1 |
| | (b) 25 cc. of suspended precipitate | +7 cc. N/10 acetic acid | 10 | 0 |
| | (c) 25 cc. of suspended precipitate | +5 cc. N/10 Na ₂ CO ₃ solution | 10 | 0 |
| | (d) Supernatant liquid | None | 10 | 0 |
| | (e) Supernatant liquid | Diluted 1 to 10 | 10 | 0 |
| 3.. | (a) Precipitate suspended in 0.5 per cent agar solution | None | 10 | 1 |
| | (b) 25 cc. of suspended precipitate | +6 cc. N/10 acetic acid in cold | 10 | 2 |
| | (c) 25 cc. of suspended precipitate | +5 cc. N/10 Na ₂ CO ₃ in cold | 10 | 4 |
| | (d) Supernatant liquid | None | 10 | 0 |
| | (e) Supernatant liquid | Diluted 1 to 10 | 10 | 0 |
| 4.. | (a) Precipitate suspended in dilute agar | None | 10 | 1 |
| | (b) 25 cc. of suspended precipitate | +5 cc. N/10 acetic in cold, pH 4.8 | 10 | 2 |
| | (c) 25 cc. of suspended precipitate | +5 cc. N/10 Na ₂ CO ₃ in cold, pH 7.2 | 10 | 2 |
| | (d) Supernatant liquid | None | 10 | 0 |
| | (e) Supernatant liquid | Diluted 1 to 10 | 10 | 1 |
| 5.. | (a) Precipitate suspended in aqueous trypsin extract and let stand over-night in ice box | None | 10 | 5 |
| | (b) Precipitate suspended in aqueous pepsin solution 3 hours | None | 10 | 5 |

* The blanks used in these experiments were all healthy at the end of the respective tests.

cated in the table, these attempts were not successful. The dilutions of the supernatant liquid, 1 to 10, were made in order to reduce the concentration of the safranin to the point where it was known that it would not inhibit the activity of the virus. It was found by trial that juice from diseased plants treated at the rate of 1 cc. of a 1 per cent aqueous solution of safranin to 50 cc. of juice remains infectious. It is necessary to add an excess of the safranin solution to complete the precipitation.

ROBERTSON (14), HOLZBERG (10), and MARSTON (11) have shown that safranin forms a rather insoluble precipitate with proteolytic enzymes. The safranin precipitate of the virus was treated, therefore, with a trypsin and also with a pepsin solution. By means of these treatments the virus was apparently displaced from its union with the safranin (see experiment 5, table II).

A means of removing the safranin from the safranin-virus precipitate, without inactivating the virus as it was set free, was then sought. After some preliminary tests it was found that picric acid forms a very insoluble precipitate with safranin. When 2 cc. of a saturated aqueous solution of picric acid is added to 25 cc. of a 0.16 per cent aqueous safranin solution, the precipitation is practically complete.

In attempts to free the virus from the safranin precipitate with picric acid, the following procedure was followed:

To 50 cc. of juice from diseased plants was added 15 cc. of a 1 per cent aqueous safranin solution; solution then placed in ice box several hours, preferably overnight, and centrifuged

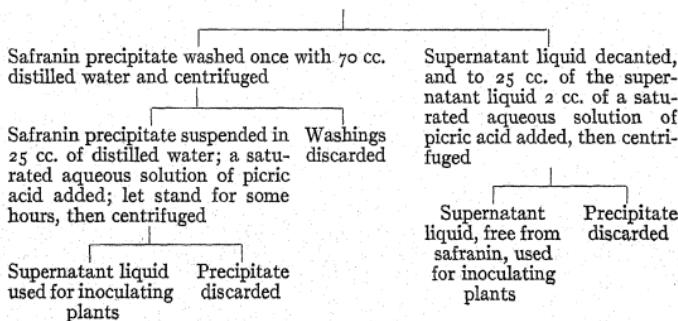


Table III gives the results obtained in freeing the safranin-virus precipitate from safranin. Experiment 5 indicates that the picric

TABLE III
LIBERATION OF VIRUS FROM SAFRANIN PRECIPITATE BY MEANS OF PICRIC ACID

| EX- PER- IMENT NO. | PREPARATION | pH OF SOLUTION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|--|-------------------|-------------------------------------|-----------------|
| 1.. | (a) Safranin precipitate suspended in 25 cc. distilled water and 2 cc. saturated aqueous solution of picric acid added; after standing, solution centrifuged and supernatant liquid used for inoculating plants..... | 3.9 | 10 | 4 |
| | (b) 25 cc. of supernatant liquid from virus-safranin precipitate treated with 2 cc. of saturated aqueous solution of picric acid, centrifuged, and supernatant liquid, free of safranin, used for inoculating plants..... | | | |
| 2.. | (a) Safranin-virus precipitate suspended in 25 cc. distilled water and 2 cc. saturated aqueous solution of picric acid added; after standing, solution centrifuged and supernatant liquid used for inoculating plants..... | 5.5 | 10 | 0 |
| | (b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1.. | | | |
| 3.. | (a) Safranin-virus precipitate suspended in 25 cc. distilled water and 4 cc. saturated aqueous solution of picric acid and 1.2 cc. N/10 Na ₂ CO ₃ solution added; after standing, solution centrifuged and supernatant liquid used for inoculating plants..... | 4.3 | 10 | 3 |
| | (b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1.. | | | |
| 4.. | (a) Safranin-virus precipitate suspended in 25 cc. distilled water and 4 cc. picric acid and 2 cc. N/10 Na ₂ CO ₃ solution added; after standing, centrifuged, and supernatant liquid used for inoculating plants..... | 5.2 | 10 | 0 |
| | (b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1.. | | | |
| 5.. | (a) To 25 cc. of diseased juice was added 2 cc. of saturated aqueous solution of picric acid, and solution used for inoculating plants..... | 6.6 | 10 | 9 |
| | (b) 25 cc. of supernatant liquid from safranin-virus precipitate treated as in experiment 1.. | | | |
| | | 5.3 | 10 | 2 |
| | | 5.3 | 10 | 10 |

acid concentration used to remove safranin from the supernatant liquid of the virus-safranin precipitate does not affect the activity of the virus appreciably.

REMOVAL OF SAFRANIN FROM SAFRANIN-VIRUS
PRECIPITATE BY MEANS OF AMYL ALCOHOL

On removal of the dye with normal amyl alcohol from the suspension of the safranin-virus precipitate, following dispersion in acid solution, activity is recovered in the aqueous layer. Hydrochloric, nitric, acetic, and oxalic acids have been used successfully to effect the dispersion of the precipitate and release the virus. When the volume of

TABLE IV
LIBERATION OF VIRUS FROM SAFRANIN PRECIPITATE BY EXTRACTING
SAFRANIN WITH AMYL ALCOHOL

| EX- PERI- MENT NO. | PREPARATION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|---|-------------------------------------|-----------------|
| 1.. | (a) Aqueous layer, 50 cc. in volume, pH 5.8-6.0, after removing safranin from aqueous suspension of precipitate with amyl alcohol..... | 10 | 10 |
| | (b) Suspension of interfacial precipitate formed on removal of safranin with amyl alcohol..... | 10 | 7 |
| 2.. | (a) Safranin-virus precipitate suspended in 50 cc. H ₂ O and 1 cc. N/1 HCl added; pH of resulting solution 2.9; safranin removed with amyl alcohol; aqueous layer, safranin free, but not neutralized..... | 10 | 9 |
| | (b) Same as 2(a), but neutralized..... | 10 | 10 |
| | (c) Same as in experiment 1(b)..... | 10 | 10 |
| 3.. | (a) Safranin-virus precipitate suspended in 50 cc. H ₂ O and 0.35 cc. N/1 HCl added; pH of resulting solution 3.1-3.2; aqueous layer, safranin free, but not neutralized..... | 10 | 10 |
| | (b) Same as experiment 3(a), but neutralized..... | 10 | 10 |
| | (c) Same as in experiment 1(b)..... | 10 | 10 |
| | (d) Supernatant liquid from safranin precipitate after removing safranin with amyl alcohol..... | 10 | 3 |

water used to disperse the precipitate is twice the volume of the juice from which the precipitate is obtained, the addition of acid produces a clear transparent solution. Under these conditions the liberation of the virus from the dye appears to be optimal. The aqueous solution obtained following amyl alcohol washing is brown in color, resembling in appearance the original juice from diseased plants. A precipitate separates at the water-amyl alcohol interface; this precipitate is also active. The safranin-virus precipitate was obtained by adding 12 cc. of a 1 per cent aqueous safranin solution to 50 cc. of juice from diseased plants. Table IV gives the results obtained.

To discover whether the conditions for safranin precipitation would be more favorable in an alkaline medium, samples of juice from diseased plants were brought to a hydrogen-ion concentration of pH 7.2 by the addition of saturated calcium hydroxide solution. Twelve cc. of a 1 per cent safranin solution was then added to 50 cc. of the treated juice. The precipitate was removed by centrifuging,

TABLE V

PRECIPITATING THE VIRUS WITH SAFRANIN FROM JUICE AT ALKALINE REACTION, THEN REMOVING THE DYE FROM PRECIPITATE AND SUPERNATANT LIQUID WITH AMYL ALCOHOL

| EX- PERI- MENT NO. | PREPARATION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|--|-------------------------------------|-----------------|
| 1.. | (a) Safranin-virus precipitate suspended in 65 cc. H ₂ O; pH of resulting suspension 7.7; aqueous layer, following removal of safranin, with amyl alcohol..... | 10 | 10 |
| | (b) Suspension of interfacial precipitate after washing twice with water..... | 10 | 9 |
| 2.. | (a) Virus-safranin precipitate suspended in 50 cc. water, 1 cc. N/1 HCl added; pH of resulting suspension 3.0; safranin free aqueous layer, not neutralized..... | 10 | 10 |
| | (b) Same as 2(a), but neutralized..... | 10 | 10 |
| | (c) Same as in experiment 1(b)..... | 10 | 10 |
| 3.. | (a) Safranin-virus precipitate suspended in 50 cc. water and brought to pH 3.6 by adding N/1 HCl; aqueous layer, following removal of safranin with amyl alcohol, not neutralized..... | 10 | 10 |
| | (b) Same as in experiment 3(a), but neutralized..... | 10 | 10 |
| | (c) Same as in experiment 1(b)..... | 10 | 10 |
| | (d) Supernatant liquid from safranin-virus precipitate after removal of safranin with amyl alcohol..... | 10 | 2 |
| | (e) Untreated diseased juice..... | 10 | 9 |
| | (f) Diseased juice diluted 1 to 1000..... | 10 | 1 |

then washed with 50 cc. of distilled water. Table V gives the results obtained on removing the dye from the precipitate and also the supernatant liquid with amyl alcohol.

SALTING OUT EXPERIMENTS ON VIRUS OF TOBACCO MOSAIC

Salting out experiments were tried after it was found that an aqueous solution of safranin precipitated the virus, and also that under certain conditions the virus apparently will migrate in an electric field.

TABLE VI
SALTING OUT THE VIRUS FROM JUICE OF DISEASED PLANTS

| EX- PERI- MENT NO. | PREPARATION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|---|-------------------------------------|-----------------|
| 1... | (a) 34 gm. $(\text{NH}_4)_2\text{SO}_4$ added to 50 cc. juice from diseased plants at room temperature, centrifuged; precipitate dissolved in 20 cc. distilled water, and solution used for inoculating plants..... | 5 | 2 |
| | (b) Supernatant liquid diluted with five volumes of distilled water..... | 5 | 0 |
| 2... | (a) 17 gm. $(\text{NH}_4)_2\text{SO}_4$ added to 25 cc. juice from diseased plants at about 0° C. in cold; after agitating, precipitate filtered off and dissolved in 15 cc. distilled water, and solution used for inoculating plants..... | 5 | 4 |
| | (b) 5 cc. filtrate made up to 50 cc. and solution used for inoculating plants..... | 5 | 0 |
| 3... | (a) 16.5 gm. $(\text{NH}_4)_2\text{SO}_4$ added to 25 cc. juice from diseased plants at about 0° C. in cold; after agitating, precipitate filtered off and dissolved in 20 cc. distilled water and solution used for inoculating plants..... | 5 | 4 |
| | (b) 5 cc. filtrate made up to 50 cc. with distilled water, and solution used for inoculating plants..... | 5 | 0 |
| 4... | (a) 60 gm. MgSO_4 added to 100 cc. juice from diseased plants; after standing overnight at room temperature, precipitate filtered out, washed several times with saturated solution of magnesium sulphate; precipitate dispersed by pulping the filter paper in 50 cc. of water. On straining the suspension through cheesecloth a greenish brown solution was obtained which with washings measured 145 cc. This solution used for inoculating plants 2½ days after the salt added..... | 10 | 9 |
| | (b) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 100..... | 10 | 9 |
| | (c) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 1000..... | 10 | 8 |
| | (d) Aliquot of the 145 cc. obtained in experiment 4(a) diluted 1 to 10,000..... | 10 | 3 |
| | (e) Original filtrate from precipitate obtained in experiment 4(a) used for inoculating plants..... | 10 | 1 |
| | (a) 13 gm. Na_2SO_4 (anhydrous) added to 100 cc. of diseased juice; after standing 18 hours at room temperature the precipitate centrifuged out and washed several times with small quantities of a 0.3 saturated solution of sodium sulphate; precipitate then dispersed in 75 cc. of water, and solution used for inoculating plants..... | 10 | 5 |
| 5... | (b) Supernatant liquid obtained in experiment 5(a) diluted 1 to 100 and used for inoculating plants..... | 10 | 9 |
| | (a) Experiment 5(a) repeated, only variation being that after the Na_2SO_4 was added the solution was held at 32° – 33° C. for 18 hours..... | 10 | 3 |
| | (b) Supernatant liquid obtained from precipitate in experiment 6(a) diluted 1 to 100 and used for inoculating plants..... | 10 | 9 |

On attempting to salt out material from the diseased juice at about 0° C., it was found that an immediate precipitate was thrown out only near the saturation point with ammonium sulphate. It was also found that about 34 gm. of ammonium sulphate to 50 cc. of the juice from diseased plants was necessary to produce this precipitate.

Table VI gives the results of some of the experiments on salting out the virus. The plants used to test these solutions were inoculated by scratching three leaves of each plant with a sterilized needle, dropping on the solution used for inoculating, and rubbing it in with a clean cork stopper.

Juice from diseased plants when 25 per cent saturated with ammonium sulphate remained infectious. In the experiments reported in table VI the original supernatant liquid and filtrates were diluted therefore, as indicated, in order to bring the ammonium sulphate concentration below 25 per cent saturation. High concentration of magnesium sulphate is apparently quite effective in salting out the virus. A precipitate forms when juice from diseased plants is 0.3 saturated with sodium sulphate and allowed to stand. As shown in table VI, however, it is doubtful whether this precipitate contains an appreciable proportion of the virus. Work on salting out the virus was soon discontinued, as the precipitates obtained not only contained the excess salt, but also protein and much pigment.

PRECIPITATION OF VIRUS OF TOBACCO MOSAIC WITH ACETONE OR ETHYL ALCOHOL

Since ALLARD (2) had shown that strong acetone or alcohol rapidly killed the virus, the precipitation by these reagents was first tried out in a cold room in order to reduce as much as possible the injury to the virus. Table VII gives the results obtained.

The preliminary experiments on precipitation of the virus by acetone, as shown in table VII, indicated that a solution of the precipitate, thrown out of juice from diseased plants by two volumes of acetone, is highly infectious. But in order to determine more accurately the proportion of the virus precipitated by adding acetone in the ratio of two volumes to one of the diseased juice, a greater number of plants were inoculated than were employed in the preliminary experiments. The method of HOLMES (9) was employed for

TABLE VII
PRECIPITATING THE VIRUS WITH ACETONE

| EX- PERI- MENT NO. | PREPARATION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|--|-------------------------------------|-----------------|
| 1... | (a) To juice from diseased plants was added an equal volume of acetone in cold; supernatant liquid decanted and precipitate suspended in distilled water; suspension used for inoculating plants..... | 5 | 5 |
| | (b) Supernatant liquid from above precipitation used to inoculate plants..... | 5 | 1 |
| 2... | (a) To juice from diseased plants were added two volumes of acetone in cold; supernatant liquid decanted and precipitate rinsed with acetone, then absolute ether; precipitate suspended in distilled water and used for inoculating plants..... | 5 | 5 |
| | (b) Supernatant liquid passed through a hardened paper filter, and filtrate diluted 1 to 3..... | 5 | 5 |
| 3... | (a) Same as in experiment 2(a)..... | 5 | 0 |
| | (b) Same as in experiment 2(b)..... | 5 | 5 |
| 4... | (a) Same as in experiment 2(a)..... | 5 | 5 |
| | (b) Same as in experiment 2(b)..... | 5 | 0 |
| 5... | (a) To 25 cc. juice from diseased plants was added 50 cc. acetone in cold; precipitate decanted and precipitate rinsed with acetone, then ether; precipitate suspended in 13.33 per cent acetone and used for inoculating plants..... | 5 | 5 |
| | (b) Supernatant liquid from precipitate passed through gravity filter and filtrate diluted 1 to 5 (until acetone concentration was about 13.33 per cent)..... | 5 | 5 |
| 6... | (a) Same as in experiment 5(a)..... | 5 | 1 |
| | (b) Same as in experiment 5(b)..... | 5 | 5 |
| 7... | (a) Same as in experiment 2(a)..... | 5 | 5 |
| | (b) Same as in experiment 2(b)..... | 5 | 0 |
| 8... | (a) Same as in experiment 2(a)..... | 10 | 10 |
| | (b) Same as in experiment 2(b)..... | 10 | 1* |
| 9... | (a) Same as in experiment 2(a)..... | 10 | 10 |
| | (b) Same as in experiment 2(b)..... | 10 | 1* |
| 10... | (a) Same as in experiment 2(a)..... | 10 | 10 |
| | (b) Same as in experiment 2(b)..... | 10 | 0 |
| 11... | (a) Same as in experiment 2(a)..... | 10 | 10 |
| | (b) Same as in experiment 2(b)..... | 10 | 3† |
| 12... | (a) Same as in experiment 2(a)..... | 10 | 10 |
| | (b) Same as in experiment 2(b)..... | 10 | 2 |
| 13... | (a) To juice from diseased plants were added three volumes of acetone in cold; supernatant liquid decanted and precipitate rinsed with ether; precipitate then suspended in distilled water and used for inoculating plants..... | 10 | 10 |
| | (b) Supernatant liquid passed through hardened filter paper, and filtrate diluted 1 to 3, then used for inoculating plants..... | 10 | 0 |
| 14... | (a) Same as in experiment 13(a)..... | 10 | 10 |
| | (b) Same as in experiment 13(b)..... | 10 | 0 |
| 15... | (a) Same as in experiment 13(a)..... | 10 | 10 |
| | (b) Same as in experiment 13(b)..... | 10 | 0 |

* One of the blanks was also diseased.

† Three of the blanks were also diseased.

inoculation, whereby only a small amount of virus is introduced by means of fine pins. Table VIII gives the results of these experiments.

From experiments 2-15 of table VII and 3, 4, 7, and 8 of table VIII it is evident that, so far as our present methods indicate, precipitation (under certain favorable conditions) of the virus from juice of diseased plants is, for all practical purposes, complete when two volumes of acetone or absolute alcohol are added to one volume of the juice. The juice obtained from tobacco plants with mosaic disease varies not only in virus content, but also in the content of other solids. It has been observed that when apparently complete precipitation is obtained, the precipitate formed, on adding the acetone or alcohol in the cold, will settle out of solution very quickly and collect in a viscous mass in the bottom of the container. This permits decantation of the supernatant liquid without loss of the precipitate, giving a sharp separation. Under certain conditions, which are not yet entirely clear, a light flocculent precipitate may be obtained which settles slowly. This lengthens the time of contact with the reagent before the supernatant liquid can be decanted. It is also very difficult to decant the supernatant liquid completely from such a precipitate, even after it has settled, without losing some of the precipitate.

Experiments 4 and 7, table VIII, were designed to demonstrate whether loss of activity could be averted by more complete recovery of a precipitate which settled slowly as flocculent material. In this experiment the precipitate was thrown down in a compact mass by centrifuging. The mother liquor was decanted completely without loss of precipitate. Recovery was about complete, in contrast to experiment 2, table VIII, where a light flocculent precipitate was obtained and was not centrifuged. A greater concentration of acetone or alcohol will induce more prompt settling of the precipitate. We prefer, however, to work with the lowest concentration that will produce complete precipitation, as the rate of inactivation must be higher, the higher the acetone or alcohol content. We prefer acetone to alcohol in precipitating the virus, as the precipitate settles much better from acetone solution, and can be redispersed more readily in water.

TABLE VIII

COMPARISON OF AMOUNT OF VIRUS IN ACETONE OR ALCOHOL PRECIPITATE
WITH THAT IN ORIGINAL JUICE SAMPLE

| EX- PERI- MENT NO. | PREPARATION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|---|-------------------------------------|-----------------|
| 1... | (a) 35 cc. juice from diseased plants made up to 100 cc. with C.P. acetone at room temperature, and allowed to stand a few minutes; precipitate obtained on centrifuging dispersed in water, made up to 100 cc., and solution used for inoculating plants..... | 150 | 54 |
| | (b) 35 cc. juice from diseased plants used in 1(a) diluted to 100 cc..... | | |
| 2... | (a) 100 cc. juice from diseased plants, at about 0° C., added 200 cc. of C.P. acetone at -15° C.; precipitate did not settle, so about 50 cc. more acetone added, then supernatant liquid decanted; precipitate suspended in water and made up to 100 cc.; 50 cc. of this solution diluted to 100 cc. and used for inoculating plants..... | 150 | 74 |
| | (b) 50 cc. of juice from diseased plants used in 2(a) diluted to 100 cc..... | | |
| 3... | (a) To 100 cc. of juice from diseased plants, at about 0° C., 200 cc. of C.P. acetone at about -15° C. was added; on mixing precipitate settled at once, giving good separation; supernatant liquid decanted, precipitate suspended in water and made up to 100 cc.; 50 cc. of this solution diluted to 100 cc. and used for inoculating..... | 150 | 58 |
| | (b) Diseased juice diluted with equal volume of water and used for inoculating..... | | |
| 4... | (a) Two volumes of C.P. acetone at about -15° C. added to juice from diseased plants at about 0° C. (last few cc. of mother liquor removed by centrifuging); precipitate taken up in water and diluted to a volume twice that of juice from which precipitate had been obtained..... | 150 | 60 |
| | (b) Experiment 4(a) repeated on another sample of same juice..... | | |
| | (c) Sample of same juice used in 4(a) and 4(b) diluted with an equal volume of water..... | | |
| 5... | (a) 30 cc. of juice from diseased plants made up to 100 cc. with 95 per cent alcohol; precipitate obtained after 30 minutes at room temperature dispersed in water and made up to 200 cc..... | 150 | 43 |
| | (b) 30 cc. of the untreated juice made up to 200 cc. with distilled water..... | | |
| 6... | (a) 30 cc. of juice from diseased plants made up to 100 cc. with 95 per cent alcohol at room temperature; precipitate thrown down by centrifuging, then dispersed in about 30 cc. of 0.05 N. HCl (pH of dispersion 4.9)..... | 150 | 45 |
| | (b) Undiluted juice from diseased plants..... | | |
| 7... | (a) 33 cc. of juice from diseased plants at about 0° C. made up to 100 cc. with absolute alcohol at about -15° C.; precipitate thrown down by centrifuging a few minutes at room temperature; precipitate dispersed in 33 cc. of 0.025 N. HCl, then 33 cc. of 0.025 N. NaOH added (resulting pH 5.61)..... | 150 | 91 |
| | | | |
| | | | 55 |

TABLE VIII—*Continued*

| EX- PER- IMENT NO. | PREPARATION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|--|-------------------------------------|-----------------|
| | (b) Same preparation as 7(a) excepting that precipitate was dispersed in 66 cc. of 0.025 N. HCl (resulting pH 4.8)..... | 150 | 68 |
| | (c) Same preparation as that of 7(a) excepting that precipitate was dispersed in 66 cc. of distilled water (resulting pH 6.2)..... | 150 | 52 |
| | (d) Sample of same juice from diseased plants diluted with equal volume of distilled water (resulting pH 5.5)..... | 100 | 29 |
| 8... | (a) 33 cc. of juice from diseased plants made up to 100 cc. with absolute alcohol, then let stand in ice box 30 minutes; precipitate thrown down by centrifuging, washed with ether, then dispersed in 66 cc. of 0.025 N. HCl..... | 150 | 55 |
| | (b) Same preparation as that of 8(a) excepting that precipitate was dispersed in 66 cc. distilled water..... | 150 | 76 |
| | (c) Sample of untreated juice from diseased plants diluted with equal volume of distilled water..... | 150 | 75 |

HEAT PRECIPITABLE FRACTIONS IN JUICE
FROM DISEASED PLANTS

The work on salting out and precipitation with acetone and alcohol made it necessary to determine, if possible, whether simple proteins were connected in any way with the virus. It was soon found that juice from diseased plants contained a fraction that coagulated at about 85° C. When the temperature is raised at the rate of about 1° C. per minute in a water bath, the juice may become turbid near 70° C. The precipitate usually begins to flocculate at 85° C. or sometimes a little below, and the coagulum begins to settle before 90° C. is reached. This coagulum may readily be filtered off. It is very dark in color, and contains considerable nitrogen. The filtrate when heated to boiling gives another precipitate, but this contains only a small amount of nitrogen. Juice from healthy plants also contains these two heat precipitable fractions, but the first fraction may come down at a lower temperature than that from juice of diseased plants.

Table IX gives a comparison of the heat precipitable fractions from juice of healthy and diseased plants. Each precipitate was washed thoroughly by placing in a beaker and boiling with 100 cc. distilled water, filtering, and then repeating the operation once. As

shown from the table, there appears to be no marked difference in the nitrogen content of the two heat precipitable fractions from juice of diseased and healthy plants.

The juice remains infectious after the first fraction is removed by heating, although the virus strength is reduced. Boiling the juice renders it non-infectious.

PARTIAL REMOVAL OF PROTEIN, SULPHATE, AND PHOSPHATE

The acetone precipitate formed by adding two volumes of acetone to one volume of the juice from diseased plants contains from 20 to 25 per cent of the dry matter of the juice. This precipitate

TABLE IX
HEAT PRECIPITABLE FRACTIONS IN JUICE FROM DISEASED AND HEALTHY
TURKISH TOBACCO PLANTS

| PREPARATION | PRECIPITATE OBTAINED BY HEATING TO 85° C. | | PRECIPITATE OBTAINED BY BOILING FILTRATE, FROM FIRST PRECIPITATE, FOR 15 MINUTES | |
|---|--|----------------------|--|----------------------|
| | Weight (gm.) | Total nitrogen (gm.) | Weight (gm.) | Total nitrogen (gm.) |
| 500 cc. juice from diseased tobacco plants..... | 0.299 | 0.035 | 0.114 | 0.005 |
| 500 cc. juice from healthy tobacco plants..... | 0.276 | 0.033 | 0.189 | 0.004 |

contains the protein fraction coagulating around 85° C., as well as much inorganic sulphate and phosphate. In beginning the work of reducing the dry matter content of the juice, and freeing the virus from more contaminating material, the first point of attack was the protein fraction just mentioned. Fractional precipitation with acetone was unsuccessful in separating the protein from the virus, as 25, 30, 35, 40, and 45 per cent acetone concentration failed to remove either all of the protein or a major part of the virus. It was finally found that most of the protein could be removed with basic lead acetate at a concentration which apparently left the virus unharmed. The solution employed was made by dissolving 200 gm. of Horne's basic lead acetate preparation in 1000 cc. distilled water. This solution is added at the rate of 19 cc. to 500 cc. of the juice from diseased plants. Extensive inoculation experiments have demonstrated that

this concentration of the lead acetate apparently does not reduce the virus concentration; but it does remove most of the protein, some phosphate, and, fortunately, much pigment. After treatment with lead acetate it was found that the acetone precipitate from the cleared juice contained considerable phosphate and sulphate. Barium acetate was then used to remove some sulphate and more phosphate. As much as 10 cc. of a saturated aqueous solution of barium hydroxide with 4 cc. of N. acetic acid have been added to 25 cc. of the juice without apparently destroying a great proportion of the virus. Following the clearing with lead, 20 cc. of a saturated aqueous solution of barium hydroxide with 8 cc. of N. acetic acid were added to 500 cc. of the original juice. Freshly expressed juice from diseased plants has a hydrogen-ion concentration of pH close to 7.0 following lead clearing; consequently, it is necessary to add acid with the barium hydroxide in order to keep the pH on the acid side. The use of 8 cc. of N. acetic acid with 20 cc. of the saturated barium hydroxide solution at 20° to 22° C. will bring the hydrogen-ion concentration of the juice to about pH 6.4 to 6.7.

It has been the practice to allow the juice to stand in a cold room overnight to freeze, following addition of the basic lead acetate solution, and also following the precipitation with an acid solution of barium acetate. In this way solutions showing only slight opalescence are obtained.

A number of preliminary experiments, using sets of ten plants each, and inoculating by the usual method of scratching the leaf surface and then rubbing in the liquid used for inoculating, gave just as many plants diseased from juice that had been treated with the lead and barium solutions as were obtained by inoculating with a sample of untreated juice. To estimate the amount of virus in this fraction the method of HOLMES (9) was employed, and table X gives the results obtained.

PRECIPITATION OF VIRUS FROM JUICE TREATED WITH LEAD ACETATE AND THEN BARIUM ACETATE

After precipitation with basic lead acetate and barium acetate, a flocculent precipitate forms when two volumes of acetone are added to one volume of such treated juice, at about 0° C. This precipitate

settles slowly and never permits complete decantation of the supernatant liquid without loss. After concentrating the treated juice (in vacuo with the bath at 50° C.) to 0.4 of the volume of the original juice taken, a precipitate is obtained when two volumes of acetone are added to one volume of the concentrated solution. This precipitate collects and settles at once, adhering to the bottom of the container. The supernatant liquid may then be decanted immediately

TABLE X

CLEARING JUICE FROM DISEASED TOBACCO PLANTS BY TREATMENT WITH
LEAD ACETATE AND BARIUM ACETATE

| EX- PERI- MENT NO. | PREPARATION | NO. OF PLANTS INOCU- LATED | NO. DISEASED |
|-----------------------------|--|-------------------------------------|-----------------|
| 1.. | (a) To 25 cc. juice from diseased plants was added 0.95 cc. of Horne's basic lead acetate solution* and centrifuged; to the supernatant liquid from basic lead acetate precipitation was added 1 cc. of saturated aqueous solution of barium hydroxide with 0.4 cc. N. acetic acid and centrifuged; aliquot from the supernatant liquid, following precipitation with $\text{Ba}(\text{OH})_2$, diluted with equal volume of distilled water and solution used for inoculating plants | 150 | 80 |
| | (b) Sample of untreated juice, same as used in preparing 1(a), diluted with equal volume of distilled water and this used for inoculating plants | | |
| 2.. | (a) Same as in experiment 1(a), except that another sample of juice from diseased plants was used | 150 | 57 |
| | (b) Same as in experiment 1(a), using aliquot of untreated juice from same sample as that used in preparing 2(a) | | |

* See text for description.

and drained off completely. More dry matter is eliminated in the latter than in the former case.

Each step in the procedure toward freeing the virus fraction from other solids has been checked numbers of times by the rough method of inoculation previously mentioned, using ten plants in each test. We have tested the activity of the acetone precipitate, from treated juice after concentration, only once by the method of HOLMES (9). Table XI gives the results of this experiment.

The experiment in table XI supports the results obtained with the rougher method. This experiment gives more evidence to the effect that there is little or no loss of virus accompanying precipita-

tion with basic lead acetate, followed by precipitation with barium acetate. For some time we were doubtful as to the effect on the virus

TABLE XI

PRECIPITATING VIRUS WITH ACETONE FOLLOWING CLEARING OF JUICE WITH
LEAD ACETATE AND BARIUM ACETATE

| PREPARATION | NO. OF PLANTS INOCULATED | NO. DESEASED |
|--|-------------------------------------|--------------|
| 19 cc. of basic lead acetate solution added to 500 cc. juice from diseased plants, mixed, placed in cold room overnight; next morning thawed, centrifuged | | |
| Supernatant liquid, decanted, then added 8 cc. of N. acetic acid in 20 cc. saturated aqueous solution of barium hydroxide, mixed, then placed in cold room again overnight; next morning thawed, centrifuged | Precipitate discarded | |
| Supernatant liquid, decanted into distilling flask and concentrated to little below 200 cc. | Precipitate discarded | |
| Concentrated, decanted into beaker and made up to 200 cc.; placed in cold room and when freezing 400 cc. of C.P. acetone added (redistilled over soda lime) at about -15° C.; precipitate falls to bottom at once; supernatant liquid decanted completely | Distillate discarded | |
| Precipitate suspended in about 40 cc. of distilled water; centrifuged | Supernatant liquid discarded | |
| Supernatant liquid decanted, and made up to 50 cc. | Slight amount of sediment discarded | |
| 1.25 cc. of this solution made up to 25 cc. with distilled water, and solution used for inoculating plants (this volume represents a dilution of original juice with equal volume of water)..... | 150 | 71 |
| Aliquot from same original juice diluted with equal volume of water, and solution used for inoculating plants..... | 150 | 68 |

of concentrating in vacuo below 50° C., but now it seems that the loss here also is small.

NITROGEN, DRY MATTER, AND ASH CONTENT
OF VARIOUS FRACTIONS

To determine the effect of lead and barium treatment, and precipitation with acetone on the nitrogen, dry matter, and ash content of the fraction containing the virus, the determinations shown on pages 34 and 35 were made, and are representative of the results we have obtained.

The figures in the procedure outlined show that the acetone precipitate obtained, after lead and barium treatment, contains about 10 per cent of the dry matter of the original juice. Present experience indicates that the nitrogen content of juice from diseased plants exceeds that from healthy plants; while the dry matter of juice from healthy plants usually exceeds that from juice of diseased plants. In our system of fractionation, only samples of juice from diseased plants give the immediate precipitate with acetone, following concentration to 0.4 the volume of the original juice sample after lead and barium treatment. No sample of juice from healthy plants has been found to give the characteristic precipitate with acetone at this point in the procedure. A turbidity forms when the two volumes of acetone are added to the concentrated solution, following lead and barium treatment of healthy juice; but an appreciable precipitate settles to the bottom of the beaker only at the end of 10 or 15 minutes.

Preliminary experiments in inoculation, after treatment of the sample with hydrogen sulphide and following a second precipitation with acetone, indicate that the solutions obtained are quite active. We have not, however, made a quantitative study by the method of HOLMES at this point in the procedure.

Preliminary experiments indicate that a solution of the second acetone precipitate is inactivated when incubated with trypsin, or more especially by a combination of trypsin and pepsin; pepsin alone seems to have no effect on the virus.

Discussion

The present concepts of the nature of the virus of mosaic disease of tobacco are based, in part, on the following: the resistance to inactivation in 2 per cent phenol solution, 2 per cent creolin solution,

| | |
|--|--------|
| Juice from diseased plants, freshly expressed: | gm. |
| Total nitrogen per 500 cc..... | 0.252 |
| Dry matter per 500 cc..... | 12.172 |
| Ash per 500 cc..... | 5.650 |

500 cc. of this juice plus 19 cc. of Horne's basic lead acetate solution placed in cold room overnight, thawed, centrifuged

Supernatant liquid, decanted, and added 8 cc. of N. acetic acid in 20 cc. of saturated aqueous solution of barium hydroxide; placed in cold room overnight, thawed, centrifuged

Precipitate discarded

Supernatant liquid, decanted into distilling flask and concentrated in vacuo to a little less than 500 cc.

Precipitate discarded

Concentrate, decanted into 500 cc. volumetric flask and made up to the mark (excess lead not removed here)

Distillate discarded

420 cc. concentrated to little less than 140 cc. in vacuo

80 cc. used for analyses:
total nitrogen in the 500 cc... gm. 0.177
dry matter in the 500 cc..... 10.365
ash in the 500 cc..... 4.376

Concentrate, placed in cold and when freezing two volumes of C.P. acetone added at -15°C .

Distillate discarded

Precipitate suspended in 50 cc. distilled water, centrifuged

Supernatant liquid discarded

Clear supernatant liquid, transferred to 200 cc. volumetric flask and made up to volume

Slight residue

120 cc. + H_2S centrifuged

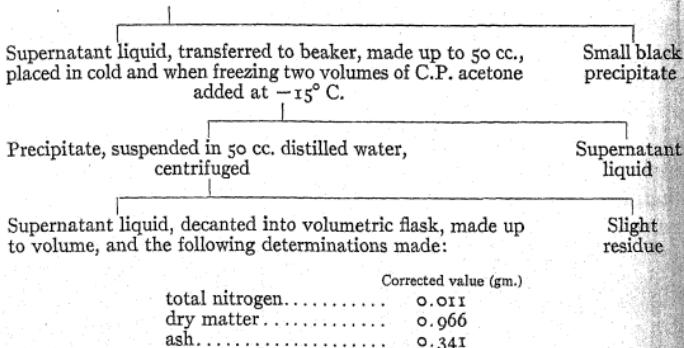
80 cc. used for analyses:
total nitrogen..... gm. 0.018
dry matter..... 1.343
ash..... .469

Supernatant liquid, concentrated in volume to less than 50 cc.

Small black precipitate

Concentrate, placed in cold overnight, then thawed, centrifuged

Distillate



and 1 per cent phenoco solution (2) is greater than that of vegetative forms of pathogens. The retention of activity in 50 per cent alcohol and 40 per cent acetone, accompanied by a complete loss of activity in higher concentrations (2), indicates less stability toward these reagents than cultures containing spores of *B. subtilis* (6), which after ten days in 99 per cent alcohol or 60 per cent acetone exhibit undiminished viability. Recently MULVANIA (13) has reported that the virus is not completely inactivated when exposed to ultra violet light 30 to 45 minutes; and a faint trace of activity remains after exposure to sunlight for 36 hours. This compares with a period of one to five minutes ultra-violet irradiation and one to two hours' exposure to sunlight required to kill bacteria either in vegetative or spore form. MULVANIA has summarized data to show that the virus of mosaic disease of tobacco is inactivated by a temperature intermediate between the thermal death point of vegetative and spore forms of pathogens.

Woods (17) was the first to suggest that the virus of the mosaic disease of tobacco was enzymic in nature. FREIBERG (7), after reviewing the evidence available on its precipitation by alcohol, adsorption on talc and inactivation by high concentrations of alcohol, by low concentrations of formaldehyde and at high temperatures, also expressed the view that the active agent was an enzyme.

We have found that when precipitation of the virus is carried

out under favorable conditions, with the proper concentration of safranin, acetone, or ethyl alcohol, the precipitation is almost complete. In each case the precipitate contains practically all of the original activity of the juice, and the virus concentration in the supernatant liquid is no greater than that obtained by diluting a fresh juice sample one thousand-fold. This, together with the fact that the virus is apparently held in an inactive condition in the safranin precipitate and is released when the safranin is removed, makes it probable that the virus which we have investigated reacted as a chemical substance.

Summary

1. An aqueous solution of safranin precipitates the virus of tobacco mosaic from juice of diseased plants. This precipitate brings down practically all of the virus. The virus is apparently held in an inactive condition in the precipitate, but is released when the safranin is removed by means of amyl alcohol.
2. Material, which gives an infectious solution when redissolved in water, has been salted out of infectious juice with ammonium sulphate and also with magnesium sulphate.
3. Two volumes of acetone or alcohol, when added to one volume of juice from diseased plants, at about 0° C., throws down a precipitate which contains practically all of the virus.
4. Juice from Turkish tobacco plants with mosaic disease contains two well defined heat precipitable fractions. One comes down around 85° C. and the other above 90° C. Only the first fraction contains an appreciable amount of nitrogen. The juice remains infectious after removal of the first fraction, although the virus concentration is greatly reduced.
5. Some of the phosphate, sulphate, and most of the protein and pigment may be removed, from juice of diseased plants, with low concentrations of lead acetate and barium acetate without apparently removing or injuring the virus.
6. Juice from diseased plants, after clearing with lead acetate and barium acetate, may be concentrated in vacuo, below 50° C., without apparent injury to the virus.
7. Cleared juice from diseased plants concentrated in vacuo to

0.4 the original volume and brought to about 0° C. gives a precipitate when two volumes of acetone at -15° C. are added. This precipitate contains only about 10 per cent of the solids of the original juice, but apparently contains all of the original virus.

8. The behavior of the virus is in many ways analogous to that of a chemical substance.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

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LOCAL LESIONS IN TOBACCO MOSAIC¹

FRANCIS O. HOLMES

(WITH ELEVEN FIGURES)

Introduction

The literature concerned with the virus diseases of plants repeatedly emphasizes the systemic nature of these infections. MAYER (6), in his original description of tobacco mosaic in 1886, stated that symptoms do not develop on the leaf inoculated, but appear on all of the young developing leaves. Essentially the same account of the course of the disease was given by BEIJERINCK (2) in 1898, by IWANOWSKI (5) in 1903, by ALLARD (1) in 1914, and by others in more recent years.

No detailed descriptions of local lesions developing at the points where tobacco mosaic virus has been introduced have been published. This has probably been due in part to the fact that the local lesions are not conspicuous in commercial tobacco, *Nicotiana tabacum*, which has been used extensively. Another factor which has made the recognition of the local development of the disease more difficult has been the practice of inoculation by scratching and severe wounding. This tends to obscure the primary lesions by producing dead areas mechanically.

A few references in the literature indicate that local lesions have been observed, although their real nature has not been understood. ALLARD (1) referred to *N. langsdorffii* as follows:

Plants of this species when inoculated through the stalk and petioles seem particularly susceptible to a very destructive and progressive rot, which begins at the point of inoculation and finally kills the plant by slowly involving the surrounding tissues. *Nicotiana viscosum* (*N. glutinosa*) is sometimes killed in exactly the same manner.

FERNOW (3) said of *N. rustica*: "The leaves generally turn yellow and then brown near each point of inoculation." The briefness of

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

these references to the local lesions developing at the site of inoculation with tobacco mosaic virus indicates that these lesions were not recognized as symptoms of the disease.

There is in the literature one account of a plant virus which produces lesions at the point of inoculation. This is the virus of ring-spot of tobacco, which PRIODE (7) describes as forming typical rings of necrotic tissue around needle punctures used to introduce it into tobacco and petunia. These local lesions are exactly like the lesions later produced when the disease becomes systemic; but tobacco mosaic has never been shown to act in this way. Most writers have specifically stated or implied that the inoculated leaf if fully developed never shows symptoms of the infection.

In the present paper the local lesions caused by the introduction of tobacco mosaic virus into the tissues of a number of *Nicotiana* species will be described, and the usefulness of these lesions in measuring the concentration of mosaic virus samples will be shown.

In a former paper (4) the writer has described a method of inoculating plants by means of very small needle punctures. This method is well suited to the demonstration of the occurrence of local lesions. The absence of extensive dead tissue around such needle punctures when transmission does not take place makes the slightest deviation from the normal condition of the leaf tissues conspicuous. The present study was undertaken because evidence was obtained that changes take place at the site of inoculation of tobacco mosaic virus into *N. tabacum*. In this plant it was noticed that pale yellow areas sometimes develop around one or more of the five pin pricks made in each plant. Since in some cases a very dilute virus was being measured, not many plants were expected to take the disease. This fact made the pale yellow areas particularly noticeable, and led to a later examination of the plants which showed them. It was found that every plant which showed such local changes around one or more of the pin pricks developed mottling within a few days. Most of the plants which did not show pale yellow areas near the inoculation punctures remained healthy. This suggested that these yellow areas might represent localized symptoms of a primary infection, since they were consistently followed by the familiar systemic symptoms. Unfortunately the pale yellow areas were very incon-

spicuous and could not be detected in all cases in which systemic symptoms developed.

It seemed possible that some species of *Nicotiana* might show local symptoms more conspicuously and more consistently than *N. tabacum*. A survey of a number of species² was therefore made. These were *N. rustica*, *N. trigonophylla*, *N. plumbaginifolia*, *N. longiflora*, *N. tomentosa*, *N. suaveolens*, *N. quadrivalvis*, *N. paniculata*, *N. sylvestris*, *N. sanderae*, *N. glutinosa*, *N. nudicaulis*, *N. langsdorffii*, *N. clevelandii*, *N. acuminata*, *N. glauca*, and *N. multivalvis*. Of these species, five showed pronounced necrotic local lesions instead of pale yellow areas. These species were *N. rustica*, *N. langsdorffii*, *N. acuminata*, *N. sanderae*, and *N. glutinosa*. A description of the local lesions in each of these five species follows.

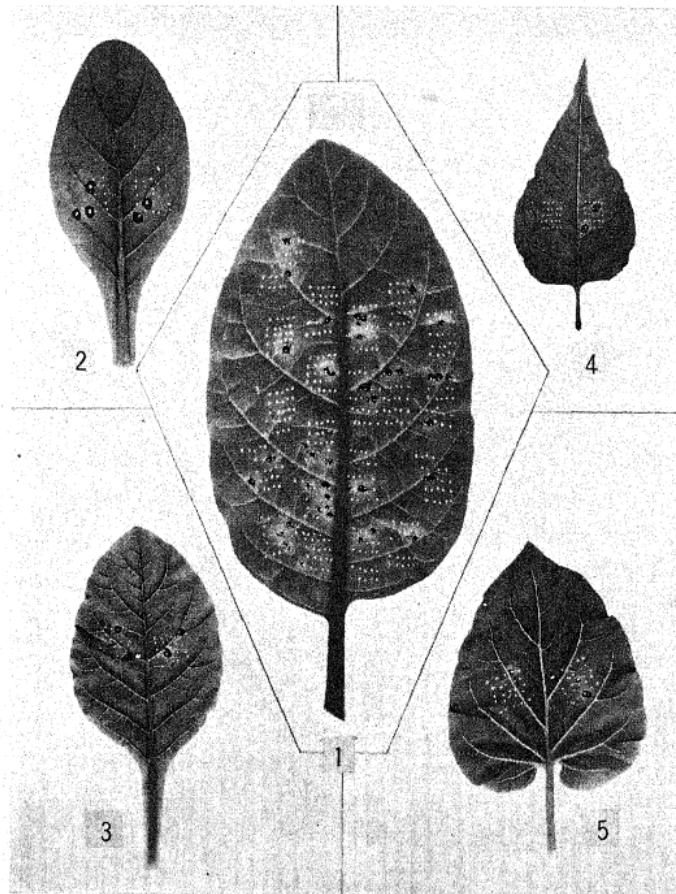
In *N. rustica*, as in all of the species studied, pin pricks which fail to transmit the virus heal perfectly with no macroscopic trace of necrosis; successful transfers, on the other hand, are marked by necrotic spots. These are easily counted eight to ten days after the time of inoculation, and may be distinguished even when many are on the same leaf (fig. 1). These necrotic spots on *N. rustica* are brown in color, circular in some cases, but frequently irregular in outline. They increase slowly in size during the first week after they appear. Later they spread rapidly, especially in young leaves. They may involve a considerable area of the leaf surface before the leaf concerned becomes old and drops off.

The lesions on *N. langsdorffii* (fig. 2) and *N. sanderae* (fig. 3) are more conspicuous than those on *N. rustica*, because they are blackish with concentric rings of dead tissue. They grow larger day by day after their first appearance, and may become very extensive. Sometimes veins and even the stem of the plant are involved in the slowly spreading necrosis of the tissues.

In *N. acuminata* (fig. 4) the lesions are irregular in outline and brown in color. They appear one or two weeks after the introduction of the virus, and increase in size rather more slowly than is the case in the other species.

² Thanks are due to Dr. S. A. WINGARD of the Virginia Agricultural Experiment Station, Dr. R. E. CLAUSEN of the University of California, and Dr. E. M. EAST of the Bussey Institution, Forest Hills, Massachusetts, for their kindness in supplying seeds of these species.

N. glutinosa exhibits a very different response (fig. 5). The lesions begin to be noticeable on the second or third day and are all completely formed by the fifth day, when in other species the



FIGS. 1-5.—Local necrotic lesions in: (1) *N. rustica*, (2) *N. langsdorffii*, (3) *N. sanderae*, (4) *N. acuminata*, (5) *N. glutinosa*. Lesions in *N. rustica* and *N. acuminata* are brown, in *N. langsdorffii* and *N. sanderae* almost black, and in *N. glutinosa* pale brown surrounded by rings of darker brown.

first indications of breakdown appear. These rapidly developing necrotic lesions appear first as tiny glistening dark spots. The centers soon dry down and develop a light brown color. Around them darker brown rings form concentrically. This gives the lesions an easily recognized appearance not to be confused with other dead spots in the leaves.

In order to discover whether these necrotic local lesions consistently appear whenever a successful transfer of the mosaic virus is accomplished, a test with small *N. rustica* plants was arranged. In a number of experiments each plant was inoculated with a single pin prick in a young leaf. The inoculation was carried out with a no. 00 black enamel insect pin previously wet with juice from a mosaic leaf of commercial tobacco. Altogether 582 plants were thus inoculated. Of these, 136 took the disease, as shown by the development of the severe systemic symptoms characteristic for this species. Every one of these plants showed a local necrotic lesion at the site of inoculation. These lesions were similar to those shown in fig. 1, except that there was but one on each plant. The fact that every plant which took the disease showed a local lesion is evidence that the local lesion is a definite symptom of the mosaic. Six plants in the series showed local lesions without a later development of the systemic disease. Apparently the virus was not able to spread in these individual cases. Their number is so small, however, that the diagnosis of transfer of the disease is not much changed if local lesions are taken as evidence of the transfer without waiting to examine the later systemic symptoms. Later experiments, in which more than one pin prick was used for each plant, have substantiated these conclusions.

Further evidence that the successful transfer of virus can be detected by examination of the local necrotic lesions is afforded by the fact that concentrated virus samples cause the development of large numbers of these local lesions when they are used as inoculum, whereas known dilutions of the virus samples in water cause the development of smaller numbers of the lesions. Graphs showing the accuracy with which the numbers of lesions are dependent upon the known concentrations of virus samples will be presented later in this paper, when the use of the local lesions in measuring virus concentrations is discussed.

No bacteria or other visible microorganisms have been found upon microscopical examination of the local lesions. Fluids from plants not affected with mosaic do not produce the lesions when used as inoculum. Yet it appeared that formal experiments ought to be carried out with virus suspensions free of bacteria to show that the production of the lesions was not due to the introduction of foreign organisms. Three samples of virus were therefore prepared by adding large numbers of a small bacterium, *Aplanobacter michiganensis*, to virus previously diluted with seven volumes of water. These three samples were then passed through Berkefeld W filter candles. The bacteria were entirely removed from the mixture, as was shown by plating from the filtrates. The virus passed through the filter with no observable decrease in its concentration. The lesions developing on *N. glutinosa* plants inoculated with these sterile filtrates were identical in appearance with those produced by the use of unfiltered samples of virus.

In further support of the view that the virus of mosaic itself is responsible for the production of these local lesions, it may be stated that exactly similar lesions have been produced by the use of a virus sample which had been frozen solid for three years, and by the use of several field samples of virus collected at widely separated points. It is improbable that any second virus or any other organism would be so closely associated with tobacco mosaic virus as to be present in each of these cases.

In order to prove beyond doubt that the lesions are not caused by any foreign organism, they should be produced in a sterile plant by the use of virus freed from bacteria. But the fact that the test plants are grown in sterilized soil from seeds seems sufficient protection against the presence of any organisms on their surface, especially as the individual plants are invariably capable of showing the lesions.

Use of local lesions in measuring virus concentrations

It has long been the custom to use the appearance of the systemic disease in *N. tabacum* plants as an indicator of the successful transfer of virus from mosaic plants or extracts from them. Several methods of this kind are in use for the more or less accurate measurement of

the relative concentrations of mosaic virus in different samples. In such work a whole plant is necessary for each successful transfer. As the accuracy of the measurement depends largely upon the number of successful transfers, the numbers of plants required usually prove a limiting factor in the number of measurements which can be made. Obviously it would be a great advantage if many successful transfers could be distinguished on a single plant or even on a single leaf. This advantage is given by the use of the local necrotic lesions here described.

It has been found that the number of successful transmissions of virus can be learned by counting the local necrotic spots at the points of inoculation in such species as *N. rustica* and *N. glutinosa* as well as by using whole plants as indicators. Moreover, since the lesions appear locally before systemic symptoms appear, economy of time is secured.

Since *N. rustica* offers the advantage of very large leaves, a method of using it for measuring virus concentrations will first be described. Dilution curves and a discussion of the accuracy attainable will be given. *N. glutinosa* has proved of great value, because on it necrotic lesions, which can readily be counted, appear very soon after inoculation. The disease may be transmitted by wiping the leaf surface gently with a cloth saturated in virus extract.

In *N. rustica* lesions are most readily counted when produced by pin prick punctures. A set of five insect pins held in a temporary handle has usually been used by the writer for introducing the virus. The pins are alternately dipped in a sample of virus and used to puncture the test plant leaf. In this way large numbers of punctures are rapidly made in a series of leaves. Usually each leaf will accommodate 250 or 500 punctures. The number of necrotic lesions developing is small in comparison with the number of punctures, but is sufficient to allow a fairly accurate reading of virus strength to be made with a few plants. In fig. 1 a leaf inoculated as described is shown. It is necessary to make inoculations of samples on opposite sides of the midvein of the same leaf or to use a large number of leaves for a single test. This is because leaves of different ages have been found to differ somewhat in susceptibility. In general the younger leaves are the more susceptible.

A curve showing the effect of dilution over a considerable range of virus concentrations is presented in fig. 6. The graph is based upon two series of experiments, one with a mosaic virus of usual

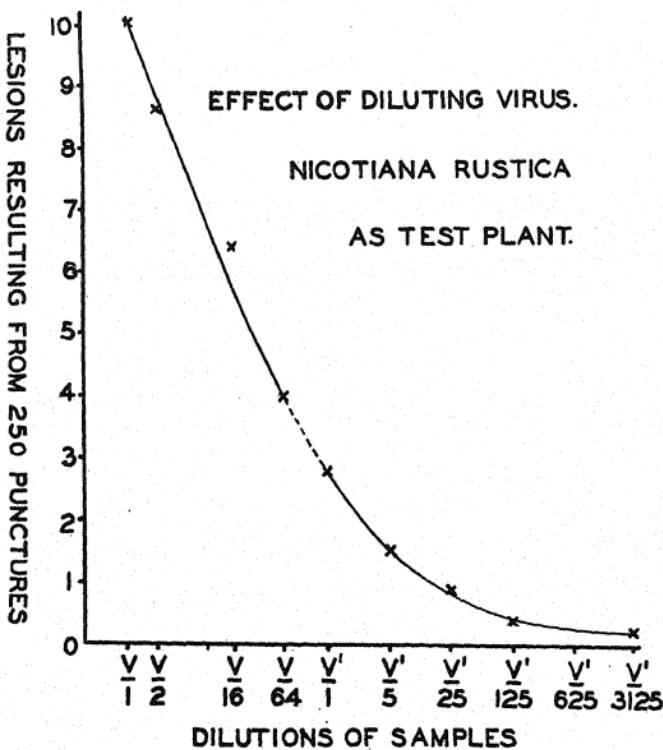


FIG. 6.—Effect of diluting virus when *N. rustica* is used as test plant. Two samples of virus, V and V' , used in obtaining the data represented. Virus V was more concentrated than virus V' , which was exceptionally weak. The lesions resulting from 38,000 pin prick punctures are represented by the nine points determining the curve.

strength and one with a weak sample. Thirty-eight thousand punctures were made to obtain the transfers represented by the figure. It will be observed that in the region in which four to ten lesions were obtained in each set of 250 punctures, the line appears nearly

straight as drawn to a semilogarithmic scale. This part of the curve is similar to that known from earlier work with commercial tobacco. It is interesting to note the direction of the line when more dilute virus is used. With *N. rustica* it has been possible to study the lower range, because greater numbers of measurements can be made by the use of local lesions than by the use of the systemic disease as an indicator of successful transmission. The upper range is the portion most frequently dealt with, and the range in which the greatest accuracy can be obtained.

Nicotiana glutinosa as test plant

The characteristics of *N. glutinosa* make it a very useful test plant for measuring virus concentrations. It has a low virus content when diseased, which gives it an advantage over *N. tabacum*, in that contaminations do not readily occur when the plants are handled. The rapidity of development of the local lesions makes it possible to have preliminary results of measurements on the second or third day after inoculation, according to the season. On the fourth or fifth day final results may be noted and the plants discarded. Large numbers of lesions can be distinguished on individual leaves; thus a high degree of accuracy may be obtained in comparing virus concentrations. The use of pin punctures is unnecessarily slow in the use of this species as a test plant. In its stead a much more rapid method of inoculation may be used, allowing tobacco mosaic virus to be measured as readily and as rapidly as bacteria are counted by plating methods.

The procedure is as follows. *N. glutinosa* plants are grown in 4-inch clay pots until flower buds begin to appear. At this stage at least five leaves on each plant are of good size. These five leaves are used for the inoculation; and for convenience in manipulation all the remaining leaves of the plant, as well as the growing point, are pinched off. This leaves a sturdy stem supporting five large leaves. Virus from any source to be tested is now taken up on a small piece of white cheesecloth and rubbed once firmly but gently over the entire upper surface of the five leaves. A full stream of tap water is used to wash away excess virus at once after this inoculation. After a little practice the operations become quite uniform. The results

justify the belief that approximate uniformity of inoculation can thus be obtained. Undiluted tobacco mosaic extracts result in the production of about 300-600 lesions on each test plant when applied in this way. If they are diluted with water, the decrease in the number of lesions is at first very rapid, later more gradual. A wide range of concentrations can be studied accurately.

As an illustration of the effectiveness of this method of inoculation, a series of leaves showing the decrease in the number of lesions appearing with decreasing concentrations of virus in the inoculum is shown in fig. 7. A dilution curve showing the effect more accu-

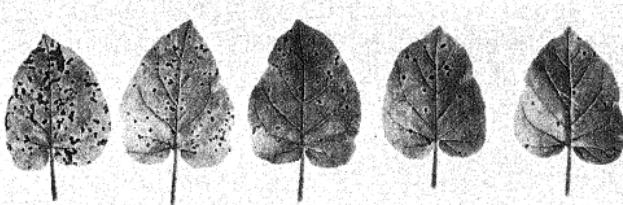
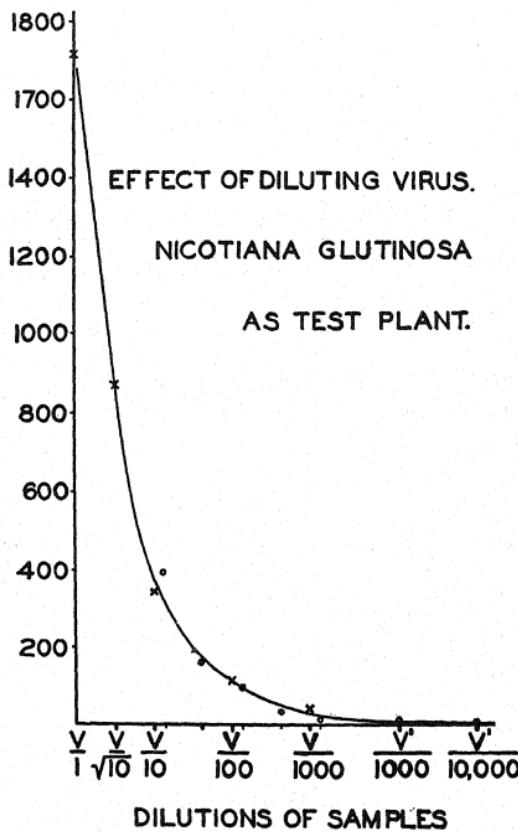


FIG. 7.—Five leaves from plants of *N. glutinosa* used to measure the effect of dilutions (1:1, 1:3.16, 1:10, 1:100, 1:1000). Numbers of lesions do not correspond to the more exact averages shown in dilution graph in fig. 8, but decrease in lesions with serial dilution is plainly shown.

rately is shown in fig. 8. Fig. 9 shows the lower range of this curve more clearly. A curve showing the probable errors of counts of lesions on single test plants is shown in fig. 10. By reference to these curves it will be observed that a very slight dilution, as by a single volume of water, can be detected with satisfactory accuracy by the use of a small number of test plants. In most preliminary measurements a single test plant is sufficient to give an excellent idea of the strength of the virus sample in hand. Even the most important measurements are usually made accurately enough if sixteen test plants are used. Fig. 11 shows two test plants, one five days after inoculation, the other one day after inoculation.

The value of this method of measuring the concentration of tobacco mosaic extracts can best be appreciated when it is compared with the old method for estimating the strength of a sample by

LESIONS



DILUTIONS OF SAMPLES

FIG. 8.—Effect of diluting virus sample, *N. glutinosa* as test plant. Counts of lesions in every case represent average number of lesions appearing for each test plant, when five leaves of each plant had been wiped with the virus sample in question. Accuracy attained may be noted by closeness of determined points to the smooth curve. Nine plants used for each determination; probable error of each point as determined is therefore one-third the error shown in fig. 10 for single observations of similar numbers of lesions.

inoculating potted plants of *N. tabacum*. Ten plants of this species were frequently used in a single test. On the average five successful inoculations and five failures would be observed on this number of test plants. On an equal number of *N. glutinosa* test plants in a

LESIONS

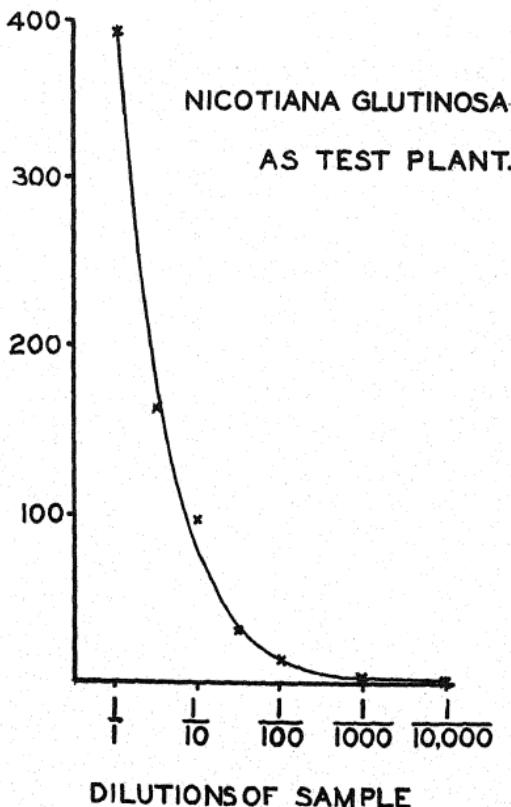
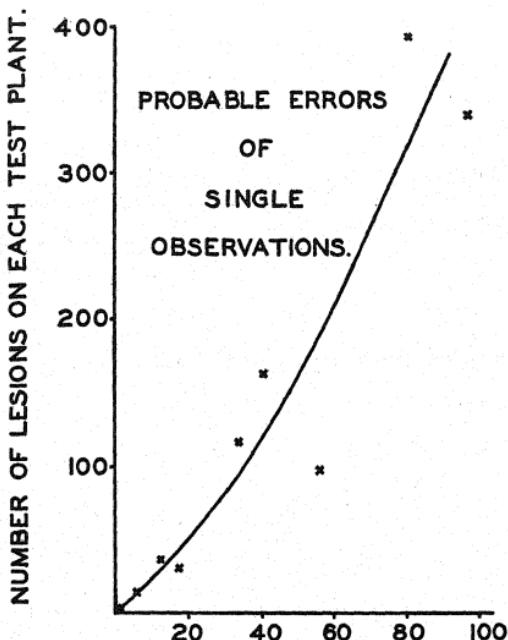


FIG. 9.—More dilute range of curve shown in fig. 8, drawn to such a scale that readings may be made accurately (see fig. 10 for probable errors of single observations in this range of measurements).

typical case 5000 successful inoculations would be observed because of the localized nature of the lesions counted. The accuracy of both



PROBABLE ERROR OF SINGLE OBSERVATION.

FIG. 10.—Probable errors of single observations for test plants having numbers of lesions between 0 and 400. The error in determining these probable errors is naturally large, and information was insufficient to extend curve beyond 400. At 1714, however, the probable error of a single observation has been found to be 414.

methods depends largely on the number of successful inoculations counted. It is estimated that in the average case for the same accuracy one test plant of *N. glutinosa* used as described serves the purpose for which at least several hundred *N. tabacum* plants are required with the customary methods of using that species.

N. glutinosa differs from the other species of *Nicotiana* in two ways. It produces very little virus when successfully inoculated, and symptoms appear on the upper parts of the plant to a very limited extent. Measurements of the amounts of virus produced by this plant have shown that the concentration present does not exceed the strength of an ordinary sample of commercial tobacco mosaic virus diluted with water to one-five hundredths of its original strength. The systemic course of the disease is not like that of most



FIG. 11.—Test plant of *N. glutinosa* (at left) as used to determine virus concentrations, inoculated five days before it was photographed. Similar plant (at right) inoculated one day before it was photographed. Lesions had not yet appeared on this recently inoculated plant.

species. Generally the growing tip soon shows the same type of symptoms which have occurred where the virus was introduced. The writer has never seen a case in which the developing leaves of *N. glutinosa* were affected with necrotic spots. The local lesions enlarge day by day, laying down ring after ring of dead tissue. After a week or so new secondary spots are sometimes formed on the peripheries of the extended primary lesions. Later lesions may appear on leaves younger than those inoculated, but they do not affect the developing leaves. Sometimes veins and portions of the stem are killed by the extending lesions. The green portions of the leaves between the primary lesions are not suitable sources of virus. No successful trans-

fers have been secured when juice from them has been used as inoculum. The virus seems to be confined to the visible lesions.

N. glutinosa, like *N. rustica*, shows a gradient of susceptibility when successively older leaves are considered. The young leaves tend to produce more local lesions when inoculated with the same source of virus as neighboring older leaves. This condition is partially remedied by removing the growing point of the plant, as is done in using the plant for measurements. In two series of plants, one with the growing tops attached, the other with the tops removed, this condition of affairs was demonstrated. In the first series, in which the growing points remained attached, the totals from top leaf to lowest leaf were 6066, 5117, 4292, 3222, and 3198. The gradient is very marked. In the second series, in which the growing points were removed, the totals were 4795, 4778, 4227, 4320, and 4343. The gradient is much less. The practice of removing the tops insures less error in comparing leaves of different age, since the chance choice of an older or a younger leaf in any plant will introduce less variation when the difference in the susceptibility is decreased.

Discussion

The recognition of symptoms which appear at the site of inoculation opens a new field of investigation in connection with tobacco mosaic. The first development of the disease seems to be very strictly localized. It is only the later course of the infection which is marked by changes in appearance of the developing leaves. The species of *Nicotiana* differ markedly in the conspicuousness of their local symptoms. In some cases necrotic spots are formed, in others a pale yellow area marks the point of entrance and early development of the virus.

Most of what is known regarding virus diseases of plants has been learned from inoculation studies. The behavior of the virus under experimental conditions, such as filtration, purification by chemical processes, and contact with disinfectants has always been judged by its reinoculation into living plants. Such tests have been difficult to carry on with a high degree of accuracy, because of the large numbers of plants required for each determination. The use of local necrotic lesions makes it possible to recognize very large num-

bers of successful transmissions on single plants. This reduces the amount of labor in many experiments. A degree of accuracy never before possible can be attained in this way with the use of moderate numbers of plants. *N. glutinosa* lends itself particularly to use in measuring virus concentrations, since the necrotic lesions in this plant develop in from two to five days. These lesions are easily counted and their relative numbers show variations in the concentrations of samples with great clearness.

When very small numbers of lesions develop on *N. glutinosa* because of the use of highly diluted samples of virus, there occur many cases in which a plant shows a single local lesion. Under such circumstances it seems possible that the infection has resulted from the entrance of a single virus particle. Transfers from this type of plant may be assumed to develop virus produced from this single particle. The process of isolation may be repeated if desired. Possibly a pure line of the causal agent is thus secured. Such single particle strains have not yet been carefully studied; their isolation and use may be expected to furnish material for future work. The use of *Nicotiana* species which show local necrotic lesions is as helpful in the study of tobacco mosaic virus as KOCH's plate method is in the study of bacterial cultures.

Summary

1. Five *Nicotiana* species were found to develop necrotic lesions wherever virus of the common field type of tobacco mosaic successfully entered leaf tissues. These species were *N. rustica*, *N. langsdorffii*, *N. sanderae*, *N. acuminata*, and *N. glutinosa*.
2. The local lesions developing in *N. rustica* can be used to measure accurately the potency of tobacco mosaic virus.
3. The local lesions in *N. glutinosa* are exceptional in the rapidity of their development. They sometimes begin to appear thirty hours after inoculation. In four or five days they are well developed. Large numbers of them may be distinguished on a single plant. This allows comparisons to be made between virus samples, since the number of lesions developing depends on the virus concentration of the inoculum.
4. A standardized method for using *N. glutinosa* as a test plant for measuring the concentration of mosaic virus gives as rapid and as

accurate results as the determination of bacterial numbers by plating methods.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

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INOCULATING METHODS IN TOBACCO
MOSAIC STUDIES¹
FRANCIS O. HOLMES
(WITH FOUR FIGURES)

The virus of tobacco and tomato mosaic disease has usually been transferred from plant to plant in mosaic studies by scratching or pricking leaves, and wetting the injured areas with extracts from mosaic plants. As a rule the virus is gently rubbed into the wounds. This process successfully transfers the infection and has been accepted widely. Occasional failures to secure the expected results suggested the advisability of making an analysis of inoculation methods. The purpose of the present study was to ascertain which methods of introducing virus were most effective.

At the beginning of the work, the problem in hand was the simple question of the proper depth of scratch to make in the surface of a leaf before wetting the area with virus. The method used was that described in the preceding paper.² *Nicotiana rustica* plants were inoculated in the ways which were to be tested, and the numbers of lesions resulting were used as indications of the numbers of successful transfers. This process soon showed that different types of wounds were very different in effectiveness.

To determine whether shallow or deep scratches were the more effective in aiding the virus to enter the tissues, the following experiment was performed. Several series of scratches, some shallow and some deep, were made on individual leaves of the test plants. Virus was applied at once by wetting a small piece of cheesecloth and lightly wiping the surface of the scratched leaf. It was expected at the time that the virus would enter some types of wound more readily than others, and that numerous necrotic lesions would cluster around the scratch of most favorable depth. If this had been the

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N. Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

² HOLMES, F. O., Local lesions in tobacco mosaic. BOT. GAZ. 87: 39-55, 1929.

case, it would have been clear whether broken surface cells or deeper layers were the more receptive. When the lesions appeared, however, it was found that very few of them were on or near the scratches. They were well distributed over the general surface of the leaf between and around the scratches. A typical leaf from such an experiment is shown in fig. 1. Apparently the scratching had been an unimportant part of the inoculation method. The light rub-

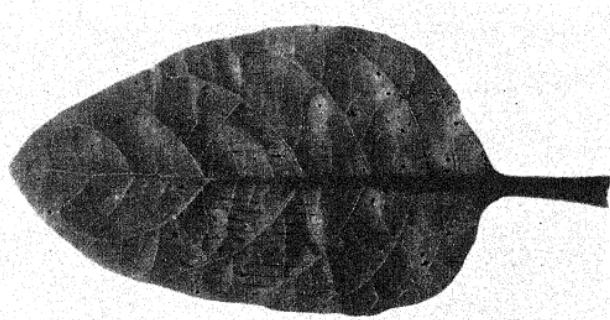


FIG. 1.—Leaf of *Nicotiana rustica* on which 20 scratches were made with needles, and virus was rubbed over the scratches and whole surface of the leaf. Resulting lesions were photographed when small, and appear as tiny dark spots, very few of which are on or near the scratches. On original leaf at time of photographing the lesions were brown, the scratches green like the general leaf surface.

bing must have produced most of the wounds necessary for the entrance of the virus.

To secure further evidence on this point another experiment was carried out. The scratching and rubbing operations were separated. On one side of the midvein of a leaf of a healthy test plant ten scratches were made. On these an extract from mosaic plants was dropped and spread with care to avoid injuring the surface near the wounded area. In the same way 200 scratches were made on twenty leaves. On the other side of the midveins the same virus was rubbed lightly into small areas of the leaves. No scratches were made here, nor was any distinct injury to be seen after the application of the virus. On a given leaf of *Nicotiana rustica* the tissues

on one side of the midvein of a leaf are precisely as susceptible as the tissues on the other side of the midvein. If the two methods of inoculation were equally effective, equal numbers of local lesions would be expected on each side of the leaves treated. When the lesions appeared, however, it was found that they were numerous on the side of each leaf into which virus had been rubbed; but they were rare or absent on the side which had been scratched and then wet with a liberal amount of the mosaic extract. On the 200 scratches only four lesions developed. Two leaves from a series of such experiments are shown in fig. 2.

A similar test was carried out to discover whether scratching through a drop of mosaic extract was effective. Twenty-five separate leaves were scratched, ten times each, through drops of the fluid. On these leaves the same virus was rubbed into similar small areas. Great numbers of lesions appeared from the rubbing, but only 13 lesions were found on the 250 needle scratches. Scratching through mosaic extract, therefore, appears slightly more effective than dropping virus on scratches, but neither method proved to be efficient as compared with light rubbing. These experiments make it seem probable that when scratching and rubbing methods of inoculation are used, the scratches are relatively ineffective, and the rubbing constitutes the effective portion of the process.

It was not possible to obtain so clear a demonstration of what happened when similar experiments were performed with commercial tobacco, because of the difficulty in detecting the spot through which infection took place. Nevertheless a test was made to see whether the response was similar. Forty-four plants of Turkish tobacco were inoculated by making ten scratches on each and immediately wetting these wounds with mosaic extract. Forty-four additional plants of the same lot were inoculated with the same mosaic extract by the simple process of rubbing lightly over a small area with a piece of cheesecloth, wetted in the same source of virus. When the plants began to show symptoms, it was found that only eight successful inoculations in forty-four trials had occurred in the group inoculated by scratching and wetting the scratches with virus. On the other hand, forty successful inoculations had resulted from the forty-four trials in the group subjected to light rubbing with

cheesecloth wet with mosaic extract. This experiment shows that *Nicotiana tabacum* behaves like *N. rustica*, in that it is not readily

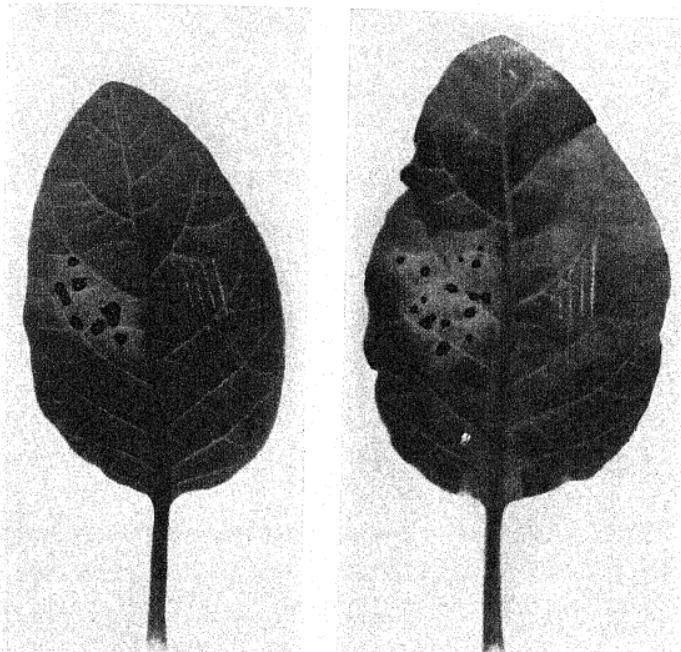


FIG. 2.—Two leaves of *Nicotiana rustica* typical of a large series in which scratches were made on one-half of each leaf and immediately wet with virus extract from a dropper; on remaining half a small area was rubbed gently with cheesecloth saturated with the same virus used to wet the scratches. Rubbing proved more effective than scratching as an inoculating method.

inoculated through scratches wetted with virus, but is more easily inoculated by light rubbing.

It seemed desirable to know whether wounds made by rubbing would be more effective when made in the presence of the virus, or whether they could be made and subsequently inoculated with equally good results. To test this a comparison was obtained in

the following way. A piece of dry cheesecloth was used to rub one-half of a leaf. Mosaic juice was then dropped at once upon this surface. The same kind of cloth wetted with the same source of virus was then used to rub the opposite half of the leaf. Lesions developed in abundance on leaf areas rubbed with cloth wet with virus, but areas rubbed first with a dry cloth and then wetted with virus developed very few necrotic spots. This is illustrated by the leaf shown in fig. 3. It was found that if a water-soaked cloth was used to break the hairs instead of a dry cloth, the result of the experiment was the

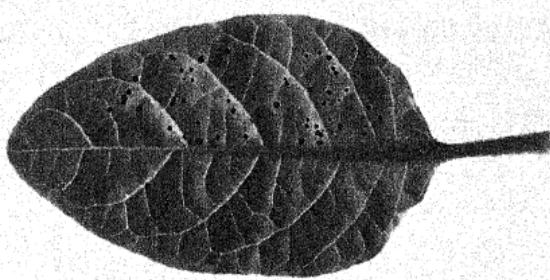


FIG. 3.—Leaf of *Nicotiana rustica* on one-half of which virus was dropped after rubbing with cheesecloth had broken the hair cells (one lesion appears); on the other half the rubbing was with cheesecloth saturated with sample of juice used on the other half. Rubbing is effective as an inoculation method only in presence of the virus.

same. The virus did not enter the wounds made before it was applied, but required wounds made in its presence.

If virus cannot readily enter wounds a few seconds after they are made, it must be that the actual entrance is completed almost instantaneously; otherwise wounds would quickly become useless although made in its presence. If the virus enters instantaneously, the practice of renewing mosaic extract above wounds and keeping it there for long periods to allow extended opportunity for entrance may be useless. To test whether it is or not the following experiment was performed. On one side of a leaf the hairs were broken with a cloth wet with water, and the area then flooded with fresh extract from a mosaic plant. On the other half of the leaf the same

kind of cloth was used, after wetting it with the same juice, to rub lightly the remaining leaf surface. As soon as the process was complete a full stream of water was used to wash the virus from this second half of the leaf, but virus was renewed over the water-rubbed portion so as to keep it wet for several minutes. Thus the first portion of the leaf was apparently favored by long contact with the virus; yet an overwhelming majority of the lesions appeared on the portion rubbed for a moment with virus and then washed clean with a stream of water. Many repetitions of this process showed the same result.

This experiment supplied additional evidence that wounds made immediately before the application of virus were ineffective. It also indicated that when virus was applied directly, it was taken up so quickly that the excess might be washed away at once without making the inoculation ineffective.

To test the possibility that the washing away of the excess virus might decrease the count to a slight extent, a quantitative experiment was performed. A group of plants were inoculated by rubbing the whole leaf surfaces with mosaic juice. As soon as each leaf was treated, one-half was washed with a stream of water. When the lesions appeared they were carefully counted for the washed and unwashed halves of the leaves. Normally the two halves of the leaves would show approximately equal counts and there would be a chance variation from side to side, but under these two treatments there was a definite response in favor of the washed portions. On the washed halves there were 1221 lesions, and on the unwashed halves 964. Nine of the twelve leaves counted showed excess lesions on the washed portions. The exceptions were nearly equal counts on three leaves. It was further noted that on eleven of these twelve leaves the lesions were slightly earlier in appearing on the washed portion. This observation lends further support to the view that the washing favored the inoculation and did not hinder it. Perhaps the water hastened the recovery of some cells by washing away the excess of toxic materials.

These experiments give some insight into what happens when virus is applied to susceptible plants. Small wounds, imperceptible to the unaided eye, take up virus instantly. Wounds made before

virus is applied are nearly or quite useless. Excess of virus may be removed at once with a stream of water without decreasing the number of effective invasions of the plant by the virus.

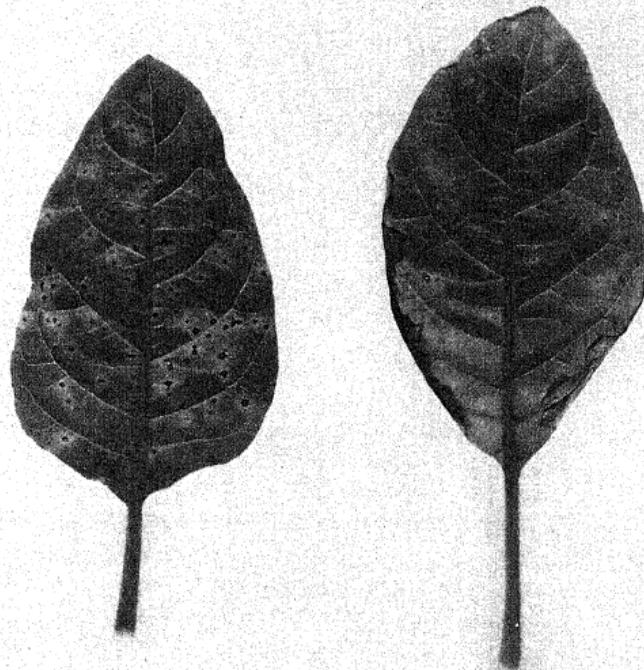


FIG. 4.—Two leaves of *Nicotiana rustica*, each of which was rubbed over its whole upper surface with cheesecloth saturated with the same sample of virus (preserved in 50 per cent glycerin). Mixture was allowed to stand on surface of leaf at right. Injury from presence of glycerin appears on edges of leaf and few lesions are in evidence. Leaf at left was washed with full stream of water at once after treatment. No injury noticeable in such cases, and abundance of lesions develop.

Practical use of this information can be made. It is often desired to treat virus with chemicals harmful to plant tissues. The virus may survive the treatment, but tests of the resulting mixture may be difficult because of the killing of the leaves by the chemicals

present. If the mixture is applied by rubbing and is then washed off with water to remove the excess, successful inoculations may be obtained.

Fig. 4 shows at the right a leaf rubbed with virus in 50 per cent glycerin. The presence of the glycerin soon caused drying of portions of the leaf and the death of certain areas. A similar leaf treated with the same glycerinated virus is shown at the left. It was washed with water at once after the inoculation. The lesions developed very much more freely in this second case, and the leaf itself showed no injury from its brief contact with the chemical. It seems probable that immediate washing of inoculated leaves when any foreign materials are applied with the virus will allow more successful transfers to be made.

Summary

1. The most effective way of transferring mosaic virus to tobacco plants has been found to be gentle rubbing over a large leaf surface with a cloth soaked in extract from mosaic plants. Scratches are much less effective, even when made in the presence of the virus.
2. The virus of tobacco mosaic does not readily enter wounds made in the leaves of healthy tobacco plants if these are made before its application.
3. Entrance seems to be instantaneous upon the production of a suitable wound in the presence of the virus. Immediate removal of the excess of mosaic extract by washing does not decrease the total number of infections, but in some cases actually increases it.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

MICROCHEMICAL STUDIES OF ROOTING AND NON-ROOTING ROSE CUTTINGS¹

MARGERY C. CARLSON

(WITH SIX FIGURES)

Introduction

Cuttings of overwintered canes of Dorothy Perkins rose produce shoots which give rise to roots when placed in humid air or in a moist medium (fig. 3). American pillar rose, treated in the same way, produces shoots but no roots, as shown by ZIMMERMAN (14). It seemed that a comparison of the anatomy and composition of such closely related plants with so marked a difference in response might give some idea of the factors influencing their rooting behavior.

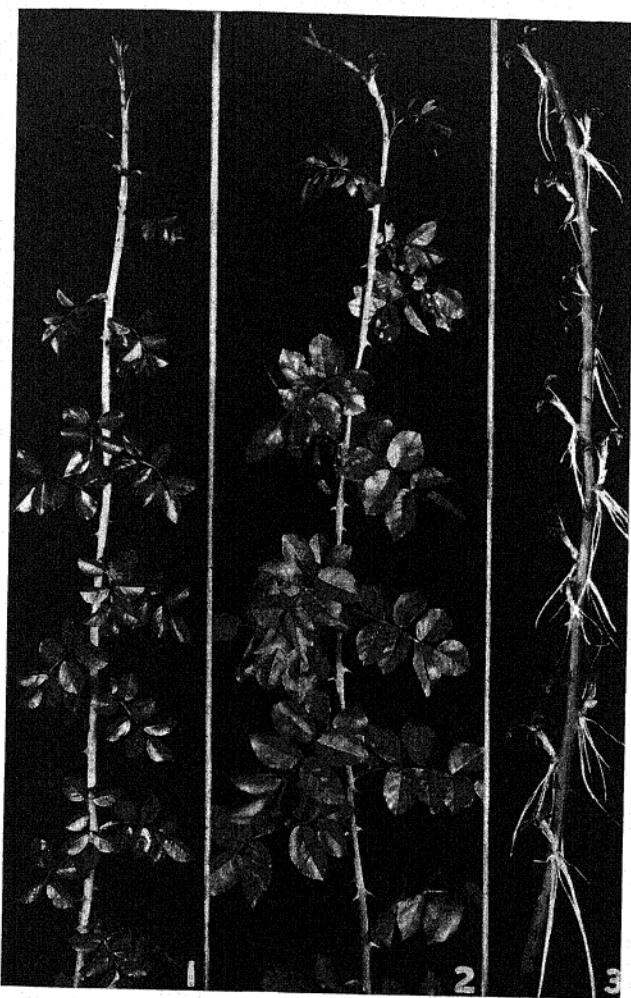
Dorothy Perkins and American pillar are hybrids with a common parent. Dorothy Perkins results from a cross of *Rosa wichuriana* × Mme. Gabriel Luizet, and American pillar from a cross of *R. wichuraiana* × *R. setigera* (1). They are climbing roses of the *multiflora* type, producing long, unbranched canes one season and flowers the second season. Figs. 1 and 2 show the vegetative canes of each rose. Dorothy Perkins has longer and more flexible canes, smaller leaves, and smaller thorns than American pillar.

Material and methods

Canes were cut into pieces 10-15 inches long, and placed with their basal ends in water in saturated atmosphere, either in a large humidity case in the greenhouse, or under bell jars in the laboratory. The humidity case was aerated only by the frequent opening of the door, but air was drawn through the bell jars by means of a filter pump.

Microchemical examinations of transverse and longitudinal sections of nodes and internodes were made at the time of collection,

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.



FIGS. 1-3.—Figs. 1, 2, vegetative canes of Dorothy Perkins (left) and American pillar (right); fig. 3, cutting of Dorothy Perkins, showing young branches and roots produced in saturated atmosphere.

and at intervals of two to four days throughout the experiments. Six series of examinations were made as follows:

| SERIES NUMBER | DATE STARTED | DURATION OF EXPERIMENT (DAYS) |
|---------------|----------------|-------------------------------|
| 1..... | March 15, 1926 | 31 |
| 2..... | March 24, 1926 | 28 |
| 3..... | April 7, 1926 | 16 |
| 4..... | April 22, 1926 | 16 |
| 5..... | March 6, 1927 | 18 |
| 6..... | April 6, 1927 | 19 |

The microchemical tests described by ECKERSON (2) were used.

STARCH.—Iodine solution in potassium iodide.

FRUCTOSE.—(1) Copper tartrate and 20 per cent sodium hydroxide, cold; (2) phenylhydrazine-hydrochloride and sodium acetate, 12 hours at room temperature.

GLUCOSE.—(1) Copper tartrate and 20 per cent sodium hydroxide, heated 1-2 minutes at 40° C.; (2) phenylhydrazine-hydrochloride and sodium acetate, heated 24-48 hours at 40° C.

OTHER REDUCING SUBSTANCES.—Copper tartrate and 20 per cent sodium hydroxide, heated 15-20 minutes at 40° C.

PROTEIN.—(1) Iodine solution in potassium iodide; (2) Biuret reaction: 5 per cent copper sulphate and concentrated potassium hydroxide.

ASPARAGIN.—Absolute alcohol on sections and identification of crystals which precipitate out.

NITRATES.—Diphenylamine in 75 per cent sulphuric acid.

CALCIUM.—(1) Five per cent sulphuric acid; (2) identification of calcium oxalate crystals.

POTASSIUM.—Platinum chloride.

MAGNESIUM.—Ammonium chloride, ammonia, and sodium phosphate.

PHOSPHORUS.—Magnesium sulphate and ammonium chloride.

TANNIN.—Ten per cent ferric chloride.

Results

OVERWINTERED CANES.—In March and April, Dorothy Perkins canes brought in from the garden contained much more reserve starch than American pillar canes. The starch was most abundant

at the nodes and decreased slightly from the base of the canes upward. In those of Dorothy Perkins the cells of the primary and secondary medullary rays, of the primary xylem parenchyma, and of the outer pith and bud gap were filled with starch. The small active cells of the inner pith were filled with starch at the nodes and partially filled in the internodes. The endodermis sometimes con-

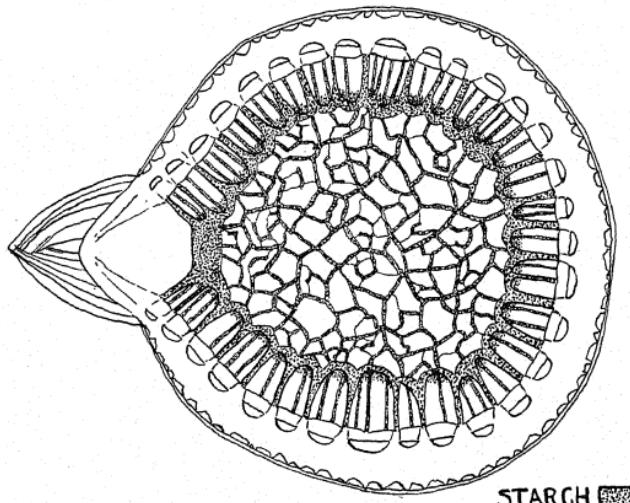


FIG. 4.—Diagram of transverse section of stem of Dorothy Perkins rose, showing arrangement of tissues and location of starch.

tained starch (fig. 4). In American pillar stems some of the medullary ray, outer pith, and bud gap cells were partially filled with starch, and the small cells of the inner pith contained a few grains each at the nodes, but were free from starch in the internodes.

There was no fructose and very little glucose in either rose. Other reducing substances (dextrin-like substances and tannins possibly) were usually present in abundance in both roses. The pith and cortex of the bud contained more of these substances than the pith and cortex of the cane. The pith of the cane sometimes con-

tained more near the bud than on the side opposite the bud. The embryonic region, leaf primordia, and procambial strands of the bud were usually free from reducing substances.

Those proteins which give the biuret reaction were present in the meristematic tissues of the bud and in the cambium and inner phloem of the cane. The cells of these tissues were filled with cytoplasm, and contained very large nuclei with large nucleoli. The pith and cortical cells of the bud also contained much protoplasm. The cells of the cambium and phloem parenchyma of the cane were high in content of protoplasm; the cells of the cortical parenchyma were lined with cytoplasm containing chloroplasts; while the small pith cells, medullary ray, and bud gap cells contained little protoplasm. The large pith cells were empty.

Tannins occurred very abundantly in the epidermis, sclerenchyma, scattered cells of the cortical parenchyma, endodermis, and in some of the cells of the bud gap and outer and inner pith.

Sphaero-crystals of calcium oxalate were more abundant in American pillar than in Dorothy Perkins. They were present in largest amounts in the pith of the bud and in the bud scales. The cortex and pith of the cane contained a considerable amount. Single crystals, probably also mostly calcium oxalate, were present in rows of cells in the sclerenchyma, collenchyma, and phloem of the cane.

The canes collected March 15, 1926, were made into cuttings and placed in water in saturated air (series 1). The changes in the content of starch and reducing substances in this series are given in table I.

All buds along the canes, except those under water, developed rapidly. As the buds grew, the reserve starch disappeared from the canes in the following order: (1) from the small cells of the pith in the internodes; (2) from the small cells of the pith at the nodes, beginning on the side opposite the bud; (3) from the outer pith and primary xylem parenchyma, beginning opposite the bud; (4) from the medullary rays, beginning opposite the bud; (5) from the bud gap.

After a few days in saturated air, a swelling appeared on the lower side at the base of the new shoots produced by cuttings of Dorothy Perkins. It usually continued to develop around the base,

TABLE I
CHANGES IN STARCH AND REDUCING SUBSTANCES IN CUTTINGS OF DOROTHY PERKINS AND AMERICAN PILLAR ROSES; SERIES I, 1926

| DATE | CONDITION OF MATERIAL | | STARCH | | Fructose | | Glucose | | TOTAL REDUCING SUBSTANCES | |
|---------------|--------------------------|------------------------|--|---|----------|----------|-----------------|-----------------|----------------------------|----------------------------|
| | Dorothy Perkins | American pillar | Dorothy Perkins | American pillar | D.P. | A.P. | Dorothy Perkins | American pillar | Dorothy Perkins | American pillar |
| March 15..... | B 2-3 mm. dormant. | B 4-5 mm. dormant. | MR + BG + P + MR + BG + P + | MR + PN + PI* + PN + MR + PN + | N* I* | N* I* | N+ I+ | N+ I+ | N+ I+ | N+ I+ |
| March 18..... | B 8-9 mm. | B 7-8 mm. | MR + BG + P + | MR + PN + P + | N* | N* | N+ I+ | N+ I+ | B +++++ | N+ I+ |
| March 20..... | | | MR + BG + P + | MR + PN + P + | N* | N* | N+ (near Br) | N+ | B +++++ | B +++++ |
| March 22..... | Br 16 mm. | Br 10-15 mm. | MR + MRN + PN + | MR + OP + P* | | | Br +++++ | Br +++++ | N+ Br +++++ | N+ Br +++++ |
| March 26..... | R just visible | R none | MR + P* | MR + (near Br) | N+ | N+ | PN + PI* + | PN + PI* + | N+ Br +++++ | N+ Br +++++ |
| March 29..... | Br 18 mm. L 4 | Br 10 mm. R 3-5 mm. | MR + MR + OP + | MR + (near Br) | N* | N+ | P + P + | P + P + | N+ N+ Br +++++ | N+ N+ Br +++++ |
| April 1..... | Br 10 mm. R 6-8 mm. | | MR + P* | MR + (near Br) | N* | N* | P + Br +++++ | P + | N+ N+ N+ Br +++++ | N+ N+ N+ Br +++++ |
| April 6..... | | | MR + BG + P* | MR + BG + P* | | | | | | |
| April 8..... | R 10 mm. | L 5 R none | MR + BG + P* | MR + BG + P* | | | N+ N+ | N+ | N+ Br +++++ | N+ Br +++++ |
| April 15..... | Br 25-35 mm. R 10 mm. | L 4 R none | MR + OP + BG + | MR + OP + BG + | | | | | Br +++++ | Br +++++ |

Letters show position on stem of materials tested: B, bud; BG, bud cap; Br, branch; I, internode; IP, inner path; L, leaf; MR, medullary ray; OP, outer path; P, path; R, root. The symbol (*) shows amount of storage material present: *, trace to none; +, very little; ++, little; +++, moderate amount; +++, considerable; +++, excessive amount.

forming a collar-like enlargement (fig. 5). Roots appeared from this enlargement after eleven days in saturated air. At this time no enlargement and no root primordia had appeared in the young shoots of American pillar cuttings.

At the time roots appeared, Dorothy Perkins canes still had much starch, only that in the cells of the inner and outer pith having

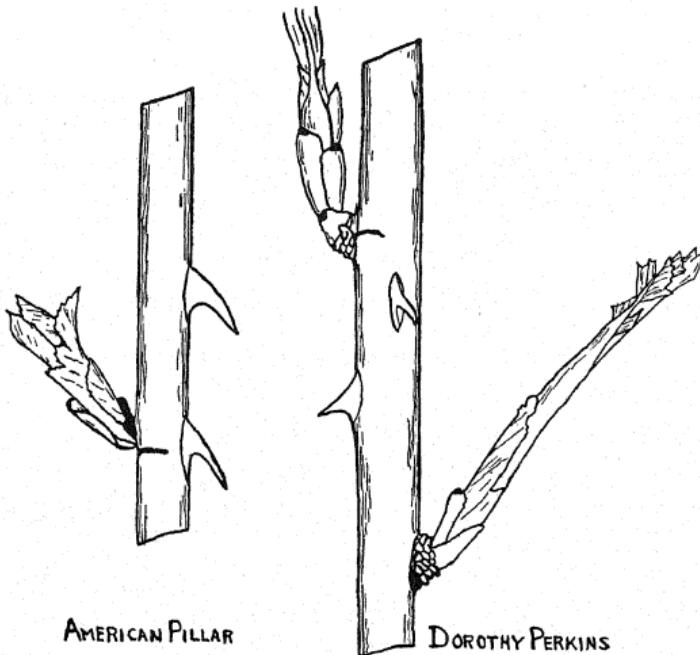


FIG. 5.—Cuttings of American pillar and Dorothy Perkins roses after 8-10 days in saturated air; roots appearing from the swelling at bases of shoots of Dorothy Perkins.

been hydrolyzed; while American pillar had lost all but a few grains in a few cells of the medullary rays, outer pith, and bud gap.

By the end of the experiment the new shoots of Dorothy Perkins were 2.5-3 cm. long, and the roots were 7-10 mm. long; while shoots of American pillar were 3 cm. long and had no roots. Dorothy Perkins still had starch in cells of the medullary rays, outer pith near

the branch, and in the bud gap; whereas all starch had disappeared from the tissues of American pillar.

No fructose was found in either rose at the beginning of the experiment. The glucose content varied in different canes, but there was usually a small amount present. After being placed in humid air the amount of glucose increased in Dorothy Perkins cuttings, and remained high until near the end of the experiment, when it dropped somewhat. Glucose was usually most abundant in the pith of the cane and young shoot. It was sometimes present in the cortex of the cane, with generally more on the side near the branch than on the opposite side. Often there was a considerable amount in the vascular cylinder, especially in the xylem vessels of the young shoot, but practically none was found in the meristematic tissues.

In American pillar cuttings the amount of glucose increased during the first few days in moist air, then decreased and remained low throughout the rest of the experiment. Glucose was high while the starch content was high, and dropped off when the starch content became low.

Reducing substances, not sugars, were present in large amounts in both roses throughout the experiment. Dorothy Perkins sometimes had more than American pillar. These substances were found chiefly in the pith of the branch and cane and in the vascular tissue.

These statements regarding reducing substances are based on the time required for reduction of copper tartrate. The osazone test, which was always made at the same time, was consistently negative for reducing sugars. This may have been due to the fact that the concentration of sugars was too low for osazone crystal formation, or to the fact that the presence of some substance (or substances) prevented the reaction. Later in the year, when the sugar content of the canes from the garden was much higher, the osazone test was positive.

The tests for nitrates were negative for both roses throughout the experiment.

Potassium was always abundant in all tissues of both roses, especially in the phloem, in the meristematic tissues, and in the pith of the branch.

The amount of calcium oxalate did not change noticeably in

either rose during the experiment. When the buds grew, the large sphaero-crystals of calcium oxalate in the pith remained at the very base of the young branches. Soluble calcium compounds were abundant in the young branches of both roses.

Proteins increased in the developing branches, but no decrease in protein content was detected in the canes. The enlargement at the base of young branches of Dorothy Perkins cuttings was due to an extraordinary development of phloem parenchyma. This tissue was high in protein content. The root primordia were first detected in this new tissue as small regions of cells which gave a pinkish color with the biuret reaction.

When kept for a few days in saturated air a dense precipitate formed with absolute alcohol in certain cells of the pith and cortex of the new shoots and in the small cells of the pith of the cane. This precipitate was not identified. After 10 or 11 days asparagin appeared in great abundance in both roses in those cells which had formerly contained the substance precipitated by alcohol. Asparagin remained high until the end of the experiments.

All of the later series gave results similar to those of series 1. In the spring of 1927, however, the difference in the starch content of the two roses was less striking than in 1926, but Dorothy Perkins again contained more than American pillar.

Fig. 6 shows the comparative changes in the starch content of the two roses in series 6. It will be seen that Dorothy Perkins canes contained considerably more starch than American pillar at the beginning of the experiment. Starch began to decrease in the canes of both roses when they were placed in humid air. In ten days roots appeared at the bases of the new shoots of Dorothy Perkins, but no roots developed on American pillar branches. On April 16, when shoots of Dorothy Perkins cuttings rooted, the canes still contained much starch at the nodes, while American pillar canes contained only a trace. At the end of the experiment, Dorothy Perkins still had a little starch, but American pillar had none.

In series 3, cuttings of both roses were placed under bell jars, some of which were covered with black paper. The temperatures in the bell jars, covered and uncovered, were the same at a given time, but varied with the room temperatures. Practically every new shoot

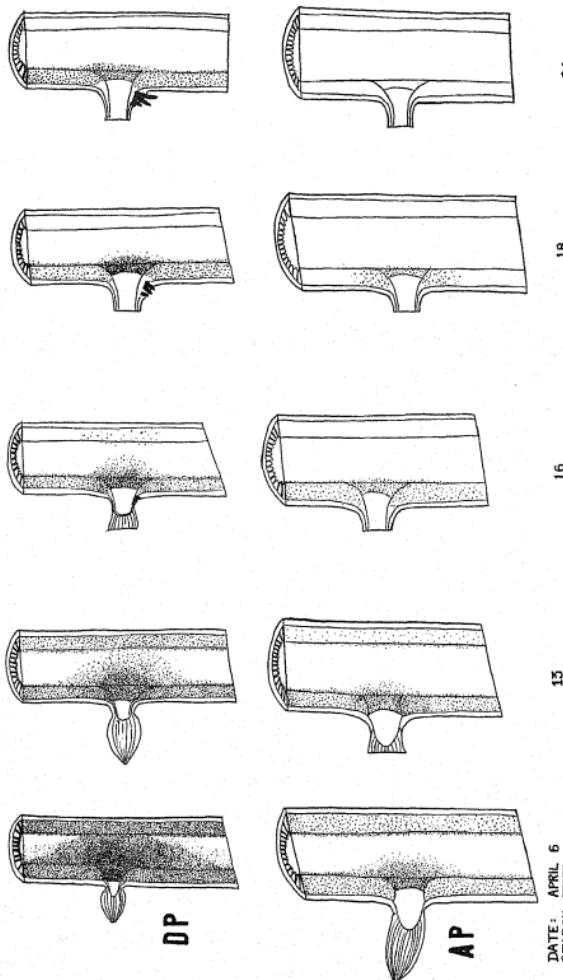


Fig. 6.—Diagrams showing comparative changes in reserve starch of Dorothy Perkins (DP) and American pillar (AP) cuttings during series 6; roots visible in Dorothy Perkins cuttings on April 16.

of Dorothy Perkins rooted in nine days, in light and in darkness; while no American pillar shoots had rooted in 18 days, when the experiment was discontinued. The changes in reserves were the same in darkness as in light.

In the spring the rooting behavior was the same, whether canes were cut into pieces or left on the plant. Canes attached to the plants were placed parallel to each other on the ground and layered with moist peat moss for a distance of about 20 inches in the middle region. The buds, which were only slightly swollen on March 28, when the experiment was started, developed normally into flower branches. On June 24 each new branch of Dorothy Perkins had a good root system at its base, while no American pillar branches produced roots.

SUMMER CANES.—The composition and rooting behavior of new canes of both roses were studied during one season. During the summer the canes elongated very rapidly. Starch was present only in the endodermis, reducing substances were high, no nitrates were detected, and calcium oxalate crystals appeared early in the pith and scales of the newly formed buds. No marked differences in the composition of the two roses were noted during their early period of growth.

American pillar canes ceased their rapid growth in September, but Dorothy Perkins canes continued to elongate until November. Starch began to accumulate in the cells of the medullary rays and outer and inner pith as growth became slower. Deposition proceeded from the base of the canes upward. The starch content of American pillar canes reached its maximum during December and then began to decrease. The maximum starch accumulation in Dorothy Perkins occurred in January and February.

Reducing sugars were more abundant in the canes while starch was accumulating than at any other time. The osazone test showed that there was an abundance of fructose and glucose in the pith, xylem, and phloem, with more at the nodes than in the internodes. After the maximum in starch accumulation had been reached, the reducing sugars were low.

New canes were made into cuttings at intervals during the summer and fall, and were treated in the same way as the overwintered canes. The upper one or two buds of each cutting developed into

branches, but the other buds remained dormant. Cuttings of both roses taken in July and August rooted from the bases of the cuttings, but not from the bases of the new shoots. As already stated, the starch content of both roses was very low during this time. Cuttings taken in the fall, when starch was accumulating in the canes, rooted from the bases of the developing branches and not from the canes. Four of eight branches of American pillar cuttings rooted, and all of eight branches of Dorothy Perkins cuttings rooted. American pillar branches produced one to four roots each, while Dorothy Perkins branches produced seven to twelve roots each. In all cases the rooting required two months for American pillar and one month for Dorothy Perkins. Unfortunately the number of cuttings used in this experiment was too small to make the results conclusive.

Experiments were performed between December and March to determine the effect of temperature on the rooting of cuttings in saturated air. Cuttings consisting of two or three nodes each were placed in constant temperature ovens at 32° , 25° , 20° , 15° , 10° , and 5° C. for one month. The results are shown in table II. As reported by ZIMMERMAN (14), rooting occurred throughout a wide range of temperature, and the time required varied with the temperature. Dorothy Perkins rooted well from the bases of young branches at 25° , 20° , and 15° , more slowly at 10° , and poorly at 32° and 5° . Rooting was equally good on pieces from bases, middles, and tops of canes. A few American pillar cuttings taken from the bases and middles of old canes rooted from the bases of young branches at 25° , 20° , and 15° C.; none rooted at 32° , 10° , or 5° . Samples of the cuttings used in these experiments were examined for starch when collected. Those cuttings which rooted contained considerable reserve starch. In experiments with summer canes, as well as with overwintered ones, there seemed to be a correlation between starch content and rooting behavior.

So far, no differences in structure of the two roses which could clearly account for their difference in rooting behavior have been detected. American pillar canes are thicker and stiffer than Dorothy Perkins canes. This is due largely to the greater amount of xylem in American pillar. The pith of Dorothy Perkins is more compact than that of American pillar.

The branches are similar in structure during their early develop-

TABLE II
Rooting of DOROTHY PERKINS AND AMERICAN PILLAR ROSE CUTTINGS AT CONSTANT TEMPERATURES

| | TEMPERATURE | | | | | | | | | | CONTROL IN LIGHT ROOM TEMPERATURES |
|--------------------------------------|-----------------|------------|-----------------|------------|-----------------|------------|-----------------|------------|-----------------|------------|---------------------------------------|
| | 3° C. | | 25° C. | | 20° C. | | 15° C. | | 10° C. | | |
| | No. of cuttings | No. rooted | No. of cuttings |
| Experiment 1, December 15-January 12 | | | | | | | | | | | |
| American pillar. | 9 | 0 | 9 | 2 | 9 | 2 | 6 | 0 | 9 | 0 | 0 |
| Dorothy Perkins. | 6 | 0 | 6 | 6 | 6 | 6 | 6 | 0 | 6 | 3 | 0 |
| Experiment 2, January 15-February 21 | | | | | | | | | | | |
| American pillar. | 9 | 0 | 10 | 0 | 9 | 0 | 12 | 1 | 10 | 0 | 12 |
| Dorothy Perkins. | 10 | 0 | 11 | 1 | 9 | 8 | 13 | 12 | 9 | 5 | 5 |

ment, but after three or four days the cambium at the base of Dorothy Perkins branches becomes unusually active toward the outside, and produces an extensive region of parenchymatous phloem tissue. The adventitious roots originate in this tissue.

Discussion

New shoots on the overwintered canes of Dorothy Perkins rose send out roots while in air of high humidity, as well as when planted in sand or peat moss. Moisture, therefore, must be the primary factor which induces root growth in Dorothy Perkins if the temperature and oxygen supply are favorable. Rooting occurred throughout a wide range of temperature, and light did not seem to play a part. Layered canes rooted as well as cuttings; evidently separation from the mother plant was not an important factor.

On the other hand, new shoots arising from overwintered canes of American pillar rose did not root. Some factor or factors other than moisture must be involved with this species. The primary difference detected between the two roses was in the reserve starch storage. Dorothy Perkins contained much more starch than American pillar in the two successive spring seasons. Winter canes kept in saturated atmosphere send out new shoots which necessarily draw on the stored food supply. The starch first disappeared from the side opposite the bud, and finally the last trace was found at the base of the new shoot. American pillar rose exhausted its entire supply in 10-12 days, whereas Dorothy Perkins rose showed a moderate amount after three to four weeks. In a few cases American pillar rooted in the fall when its starch content was high, and both failed to root when they lacked starch reserve.

Preliminary experiments to increase the carbohydrate content of American pillar canes and to decrease it in Dorothy Perkins before testing their rooting have not yet been successful.

The relation between the reserves and the rooting behavior of cuttings has been reported by other investigators. KRAUS and KRAYBILL (3) found that cuttings of tomato which are old and yellow in color, high in carbohydrates, and low in total nitrogen and nitrates, root well in humid air. Green stems which contain starch and are fairly high in total nitrogen will root, but not so profusely

as the former; and green stems without starch reserves and very low in free reducing substances but high in total nitrogen and nitrate nitrogen will not root. This work was extended and confirmed by STARRING (13), REID (7, 8, 9), and SCHRADER (10), all working with tomato.

PRIESTLEY (6) states that in some cases the food supply largely controls the production of new root initials. He thinks that adventitious roots arise in the cambium, and that stems which have no true endodermis allow leakage of food out through the cortex to the superficial meristems, with consequently not enough food remaining in the cambium for the production of root initials. Some etiolated shoots produce an endodermis. These make little growth from the superficial meristems, but root with ease, since the nutrients are held by the endodermis and can be used by the cambium.

SMITH (12), working with *Coleus*, found that the condition of the carbohydrate reserves had a marked influence on rooting. Plants in full light, with reserve carbohydrates largely in the form of starch, rooted sooner and better than plants in the shaded greenhouse where reserves were mostly reducing sugars.

Rooting from the bases of Dorothy Perkins branches involves, first, a special activity of the cambium by which an extraordinary amount of phloem tissue is produced, and second, the change in certain cells of the new tissue from the parenchymatous to the meristematic condition, thus initiating root primordia. The principles involved in these processes are still largely a matter of theory. PEARS-ALL and PRIESTLEY (5) account for the transformation of tissues from a vacuolated, non-dividing condition into a non-vacuolated, or only slightly vacuolated, meristematic condition by the fact that they lie across a gradient of hydrogen-ion concentration. Along this gradient the principal proteins of some cells are at their iso-electric point. These cells will lose water, accumulate protoplasm, and become meristematic.

SMITH (11) states:

Cell division is the expression of a series of catenary reactions depending upon the presence in a certain concentration and a certain ratio of carbohydrate and amino-radicles. Only when this carbon-nitrogen balance is maintained can a cell remain meristematic. Under normal conditions the carbohydrate is always

in excess, and it is this excess which determines cell-maturity and the cessation of new growth. If by any means the required C:N ratio can be restored, a mature tissue can be incited to regeneration.

Summary

1. Adventitious roots are produced in humid air on the new shoots from cuttings of Dorothy Perkins rose and not from like portions of American pillar rose.

2. Microchemical examination showed that Dorothy Perkins cuttings contained more reserve starch than American pillar cuttings. At the time of rooting, Dorothy Perkins had considerable reserve starch remaining, while during a similar period the reserve starch of American pillar was depleted.

3. The changes in the reserves were the same in both roses. As the starch in the canes was hydrolyzed, the content of reducing sugars increased, especially in the new branches. Asparagin was abundant in the branches after 8-11 days.

4. Only slight differences in the anatomy of the overwintered canes were noted. A swelling at the base of new branches of Dorothy Perkins was produced by an unusual development of secondary phloem. Adventitious roots were initiated in this region. These changes did not occur in American pillar branches.

5. Rooting from the bases of young shoots of Dorothy Perkins rose seemed to be related to the high content of reserve starch.

NORTHWESTERN UNIVERSITY
EVANSTON, ILL.

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GROWTH OF SEEDLINGS IN LIGHT AND IN
DARKNESS IN RELATION TO AVAILABLE
NITROGEN AND CARBON¹

MARY E. REID

(WITH PLATES I-IV)

Introduction

The influence of reserve substances in the plant upon its subsequent growth involves a multitude of problems of great importance, both from a practical and from a theoretical standpoint. The present investigations are concerned with the influence of certain types of reserve foods found in seeds upon the development of the seedling. A study has been made concerning the relation to growth of the amount and nature of the reserves of carbon and nitrogen which an embryo plant has at its disposal.

Some of the more practical questions which have prompted the research are as follows:

What are the responses of seedlings having available different amounts of reserve carbon and nitrogen when extra amounts of carbon and nitrogen are supplied externally? Is fertilization with nitrogen advisable in the early growth of all types of seedlings, regardless of weather and light conditions and the nitrogen reserves of the seed? Is the seedling with the larger reserves of carbon better able to live and thrive during prolonged periods of dull, cloudy weather during the early growth of the plant? Is the seedling with the larger amount of stored nitrogen at its disposal better able to withstand conditions of drought or of poor soil during the early phase of development, when nitrogen from an outside source is unavailable or available in very limited amounts?

Although the results of these experiments do not furnish answers to all these questions, it is considered that they supply considerable additional data toward this end. Previous investigations (11) with

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tomato cuttings varying in the relative proportions of the reserves of carbohydrates and nitrogen had shown very different responses when exposed to different environmental conditions, such as variations in light and darkness and the presence and absence of nitrate nitrogen in the nutrient medium. It was found that: (1) An abundant reserve of carbohydrates favored the rapid assimilation of nitrates. This was in agreement with the observations of KRAUS and KRAYBILL (8) in their study of the growth of the tomato plant. (2) An abundant reserve of carbohydrates but limited supply of nitrogen favored the growth of roots, but not that of shoots. (3) An abundance of nitrogen and an abundance of carbohydrates favored the growth of both shoots and roots. (4) An abundance of nitrogen but a limited amount of carbohydrates favored growth of shoots, especially of leaves, but not growth of roots. (5) The assimilation of nitrates occurred both in light and in darkness, but more rapidly in light, and the assimilated products were used in favoring growth of shoots especially.

The present studies have dealt with the development during the seedling stage only, and have related primarily to the influence of varying amounts of nitrogen and carbon upon the growth of shoots and roots. Experiments have been performed in light and in darkness with no nitrogen obtainable except that present in the reserves of the seeds, and also with nitrogen available in the form of nitrates. The following types of seeds were used:

| | | |
|---|---|---|
| Little Club wheat | Low protein content; high starch; low fat | Relatively high content of gliadin and glutenin; amide N high; relatively small amount of globulin protein |
| Vermont Champion barley..... | | |
| Giant Winter rye..... | | |
| Illinois low-protein corn..... | | |
| Hänncchen barley (Dickinson, N.D.)..... | | |
| Blue stem wheat..... | Relatively high protein content; high starch; low fat | Relatively high content of gliadin and glutenin; high-protein corn lower in amide N and higher in globulin than low-protein type (SHOWALTER AND CARR, 14) |
| Marquis wheat..... | | |
| Hänncchen barley (Aberdeen, N.D.)..... | | |
| Illinois high-protein corn..... | | |

| | |
|------------------------------|--|
| First of all peas | Moderately high protein content; moderately high starch content; low fat (except soy bean) |
| Canada White peas..... | |
| New Era cow peas..... | |
| White Marrow bush beans..... | |
| Pekin soy beans..... | Very high protein; low starch; high oil { High globulin content; amide N low |
| Mammoth Russian sunflower . | |
| Large Warted Hubbard squash | |
| Rocky Ford muskmelon..... | |
| Bonnie Best tomato..... | |

Experimental methods

The seeds were sterilized by immersion in a 0.25 per cent solution of uspulun. Starchy seeds were left in the solution for one hour, and those of the oily type for one-half hour, after which they were rinsed in freshly distilled water. The seeds were placed in germinators between layers of moist filter paper and allowed to sprout before being planted. This precaution was necessary since some of the seedlings were to be grown without any nitrogen from an outside source, and consequently the possibility of the seedlings obtaining nitrogenous products by the decomposition of seeds which failed to germinate had to be avoided. When the radicles had attained a length of 0.5-2 cm. the seedlings were planted in pulverized quartz and contained in 7-inch clay bulb pots. Glazed porcelain dishes with rims an inch high were used as saucers. The pots containing the sand, together with the saucers, had previously been sterilized by heating for an hour in a steam sterilizer maintained at a pressure of 15 pounds. The cultures were moistened with nutrient solutions made with salts of tested purity, and prepared according to the following formulas:

SOLUTION CONTAINING NITRATES

A

2 per cent $Mg SO_4$
2 per cent KH_2PO_4
2 per cent KNO_3

B

4 per cent $Ca(NO_3)_2$
2 per cent $CaCl_2$
1.5 per cent $CaSO_4$

SOLUTION LACKING NITRATES

A

2 per cent $Mg SO_4$
2 per cent KH_2PO_4
1 per cent KCl

B

2 per cent $CaCl_2$
1.5 per cent $CaSO_4$

The solutions were diluted before applying them to the cultures. In preparing the solution containing nitrates, 100 cc. of solution A was made up to 1 liter with distilled water; 100 cc. of solution B was diluted in the same manner and the solutions A and B were then mixed. The solution lacking nitrates was diluted similarly. The cultures were given fresh solutions every second day in some experiments, and every third or fourth day in others. The time for making fresh applications was determined largely by light and temperature conditions, which affected the rate of growth and the ability of the seedlings to utilize the mineral nutrients. The same procedure was followed throughout the course of any one experiment. The level of the solutions in the saucers was kept nearly constant between times of applying nutrient solutions by the addition of distilled water. Freshly distilled water was used at all times. This precaution was considered necessary, since it has been shown by SEIBERT (13) that certain kinds of microscopic organisms may thrive in distilled water tanks and pipes, and that these organisms have the ability to fix atmospheric nitrogen. The external nitrogen supply was controlled in these cultures, except that ammonia in the atmosphere was not eliminated as a factor. However, all seedlings in any experiment were subjected to the same atmospheric conditions with reference to this factor, and hence it is unlikely that it can be responsible to any extent for the differences in growth that are here described. Experiments have been conducted with seedlings grown in darkness and in light with each of the two types of solutions. Except in one case (March 5-26, 1926) all experiments were repeated, and the results of the second test were found in general to be in close agreement with those of the first.

Growth of seedlings in darkness

The question of etiolation has enlisted the interest of a number of physiologists, whose investigations on the problem were relatively extensive in the botanical literature of fifty to sixty years ago. SACHS, G. KRAUS, GODLEWSKI, STEBLER, PFEFFER, BATALIN, RAUWENHOFF, and VINES made important contributions at this time. Still earlier, DECANDOLLE (2) had described the characteristics of the etiolated plant, and attempted, as have many others

since, to account for the peculiarities of growth in darkness. The chief characteristics of the etiolated plant as mentioned by these earlier investigators may be said to be a greatly elongated stem, and leaves much reduced in size. Cotyledons which in the light grow and develop into foliage leaves remain small and undeveloped in darkness. Of all the peculiarities of the etiolated seedlings perhaps the absence of chlorophyll is the most outstanding. Almost no mention is made of the characteristics of the roots of etiolated seedlings.

The results of some of SACHS' (12) experiments show that the great elongation of the stem and reduction in size of the leaves of etiolated plants is not without exceptions. The leaves of many monocotyledonous plants become longer in darkness than in light. Also the leaves of certain dicotyledonous plants, as *Beta*, become almost as large when grown in darkness as in light. SACHS postulated that the reason leaves failed to develop in darkness was not because of lack of food (carbohydrates supposedly), but was due to some unknown influence of chlorophyll.

DE SAUSSURE (3) was probably the first to state that leaves are dependent for their growth on the products of their own assimilation (carbohydrates inferred). G. KRAUS (9) was also of this opinion, and claimed that the leaf can only develop in darkness to the stage where it can begin to assimilate if it receives the light; if it cannot assimilate it will remain small and soon die. His only proof, however, for the fact that assimilation is essential for the growth of leaves is that he did not find starch in etiolated leaves. He recognized the fact that non-nitrogenous reserve materials as fats may be present in cotyledons, and yet the cotyledons do not grow beyond a certain relatively small size in darkness. He considered that deficiencies in cell wall formation were the chief limiting factors, therefore, and that light is necessary for the transformation of materials into cell walls. His own work (9) and that of RAUWENHOFF (10), and of a number of later investigators, have shown that light has a marked influence on the thickening of cell walls and the maturation of tissues. G. KRAUS and RAUWENHOFF thought the effect was produced by the processes of synthesis and utilization of carbohydrates. GODLEWSKI (5) claimed that the influence of light on the thickening of cell walls and its growth-retarding effect on the

growth of stems have nothing to do with the assimilation of carbohydrates. VINES (16) had previously presented data which were interpreted as proving also that the retarding influence of light on the growth of leaves is completely independent of assimilation. Both of these investigators referred to the need of uniform light for the growth of stem and leaf, rather than to the effect of strong light as compared with weaker light.

Although most of the research as to the effects of light and darkness on the growth of different organs has been conducted with seedlings, not much attention has been paid to the influence of the amount and nature of the food reserves of the seed upon the type of growth. Nevertheless such a relation was partially recognized by the investigators who studied somewhat the chemical content of the seedlings. Descriptions of the growth responses are very incomplete, however.

It has not been the province of this research to enter into a detailed study of the phenomena of etiolation, but rather to obtain some quantitative measurements of the growth of different organs, to be used for comparison with results of subsequent experiments with seedlings grown in the light.

I. SEEDLINGS GROWN WITH NO EXTERNAL SOURCE OF NITROGEN

The seedlings of each kind were allowed to grow until the shoots attained their maximum length. Several preliminary tests were made to determine the most practicable method. Measurements of the heights of certain plants in each culture were made at intervals as the time of attaining maximum size approached. When no further elongation of the shoot occurred the experiment was terminated. The general appearance of the plant was taken into consideration, and slight indications of shrinking or wilting of the leaves were likewise used as an index that there was cessation of growth. The dark room in which the seedlings were grown was maintained at a nearly uniform temperature (21° C.), and favorable conditions of atmospheric moisture were obtained by evaporation of moisture from the cement floor covering the pebbles which was sprinkled with water daily.

In harvesting the seedlings, the roots were carefully washed

free of the quartz sand and rinsed in distilled water. Measurements of the lengths of roots and stems and of length and width of leaves and cotyledons were obtained, and also the green weights of the tissues. The quantitative results are given in table I, and illustrations of cow pea, soy bean, muskmelon, tomato, sunflower, and high- and low-protein corn seedlings are shown in figs. 1-7. Due to the fact that so many different kinds of seedlings were grown in these experiments, it was not possible to preserve the material for dry weight determinations.

Seedlings which grew from high-protein, high-oil seeds had the highest shoot to root ratios. This largely results from the development of an exceedingly long stem and a small amount of roots. The stems have a very high water content and a relatively small amount of protoplasm in the cells. Some of the seedlings of this group (for example, squash) may produce one leaf which remains very small, or no visible leaves, as in the case of tomato. Sections of the incompletely developed cotyledons showed that the tissues have a very dense compact structure. The cells remain small and intercellular spaces are only slightly developed. The similarity in behavior of these seedlings, representing three different families, suggests that the type of growth is related to the nature and quantity of reserve foods which are available.

The smallest weight of shoots in proportion to roots was found among the Gramineae. The low ratio results from the relatively greater weight of roots, the small size or lack of stems, and the extensive leaf development in which a large amount of the reserve materials has been used. The leaves of these seedlings have a much higher protoplasmic but lower water content than had the stems of the high-protein dicotyledonous seedlings previously described. Seedlings of the grass family have leaves longer and narrower, but thinner in cross-section, than those of similar seedlings grown in the light in normal atmosphere. This difference in length of leaf in light and in darkness results chiefly from the greater length of the leaf sheaths in the case of seedlings grown in darkness. The leaf blades of the high-protein types of the grass family tend to be longer in the light than in darkness, but those of the low-protein types tend to be longer in darkness than in light. Microchemical tests for starch

TABLE I
SEEDLINGS GROWN IN DARKNESS WITHOUT NITRATES, MARCH 7-APRIL 7, 1924

| PLANT | VARIETY | NO. OF PLANTS | LENGTH OF GROWTH PERIOD (DAYS) | TOTAL WEIGHT OF SEEDS (G.M.) | TOTAL NITROGEN IN SEEDS (%) | SHOOTS (CM.) | ROOTS (CM.) | GREEN WEIGHTS PER PLANT | | RATIO OF WEIGHTS OF SHOOTS TO ROOTS | RATIO OF GROWTH FEEDS OF RESERVE FOODS (G.M.) | TOTAL |
|-----------------|-----------------------|---------------|--------------------------------|------------------------------|-----------------------------|--------------|-------------|-------------------------|--------------|-------------------------------------|---|-------|
| | | | | | | | | SHOOTS (GMS.) | ROOTS (GMS.) | | | |
| Wheat | Blue stem | 60 | 17 | 2.32 | 2.27 | 24.0 | 8.5 | 0.151 | 0.068 | 0.084 | 2.22 | 5.6 |
| Wheat | Marquis | 60 | 16 | 2.68 | 2.50 | 27.5 | 8.9 | 0.166 | 0.054 | 0.086 | 3.04 | 6.3 |
| Wheat | Little club | 60 | 16 | 1.99 | 1.52 | 20.8 | 15.2 | 0.158 | 0.066 | 0.087 | 2.39 | 6.7 |
| Barley | Vermont champion | 60 | 15 | 1.59 | 1.82 | 21.5 | 10.2 | 0.135 | 0.062 | 0.073 | 2.19 | 7.4 |
| Oats | Storm king | 60 | 14 | 1.83 | 2.68 | 21.9 | 11.8 | 0.150 | 0.086 | 0.078 | 1.75 | 7.6 |
| Rye | Giant winter | 60 | 15 | 1.37 | 1.75 | 19.0 | 9.7 | 0.113 | 0.052 | 0.071 | 2.19 | 7.2 |
| Rice | Honduras | 60 | 20 | 1.41 | 1.68 | 34.0 | 5.6 | 0.095 | 0.031 | 0.020 | 3.08 | 5.3 |
| Corn | Illinois high-protein | 12 | 14 | 3.27 | 2.76 | 36.0 | 23.7 | 1.470 | 0.686 | 0.696 | 2.14 | 7.9 |
| Corn | Illinois low-protein | 12 | 14 | 4.06 | 1.99 | 29.2 | 26.0 | 1.290 | 0.750 | 0.575 | 1.72 | 5.2 |
| Corn | Illinois high-oil | 12 | 15 | 3.27 | | 32.9 | 23.8 | 1.402 | 0.772 | 0.728 | 1.31 | 8.0 |
| Corn | Illinois low-oil | 12 | 15 | 7.31 | | 36.1 | 27.0 | 1.800 | 0.950 | 0.766 | 1.90 | 4.5 |
| Peas | First of all | 36 | 19 | 6.36 | 4.11 | 40.5 | 9.3 | 1.121 | 0.397 | 0.067 | 2.82 | 8.7 |
| Peas | Juno | 36 | 20 | 9.46 | 5.10 | 29.0 | 9.3 | 1.268 | 0.513 | 0.131 | 2.47 | 6.7 |
| Beans | White Marrow bush | 30 | 16 | 9.99 | 3.85 | 51.3 | 13.2 | 1.993 | 0.526 | 0.111 | 3.78 | 7.6 |
| Cowpeas | New era | 26 | 13 | 3.39 | 4.40 | 32.1 | 9.6 | 0.757 | 0.237 | 0.058 | 3.49 | 7.6 |
| Soy beans | Peking | 26 | 26 | 1.75 | 3.40 | | | 0.642 | 0.172 | 0.074 | 3.72 | 12.1 |
| Mammoth Russian | 18 | 17 | 0.85 | 4.56 | 27.2 | 6.7 | 1.020 | 0.195 | 0.165 | 9.71 | 23.8 | |
| Hubbard Warted | 14 | 19 | 2.04 | 5.10 | 28.6 | 13.0 | 2.867 | 0.432 | 0.520 | 6.63 | 23.1 | |
| Red Rocky Ford | 20 | 19 | 0.26 | 5.62 | 14.0 | 7.0 | 0.251 | 0.040 | 0.040 | 6.25 | 22.4 | |
| Tomato | Bonnie Best | 33 | 15 | 0.08 | | 10.1 | 3.2 | 0.040 | 0.005 | 0.007 | 7.26 | 18.8 |

and free-reducing substances, made on tissues of some of the seedlings just previous to harvesting, showed that many of the seedlings contained little or no starch except in the guard cells of the leaves and occasional grains in the bundle sheaths at the time the seedlings stopped growing. However, some of them contained small amounts of reducing substance.

Seeds of the Leguminosae are in certain respects intermediate to the other two groups in the chemical composition of the reserve foods, and the seedlings have a tendency to be intermediate in their responses with respect to the relative proportions of shoots to roots. Several types of seedlings of this group (for example, two kinds of peas) stopped growing before the reserves from the cotyledons were depleted. Microchemical tests showed that very little reducing substance could be found in the seedlings or in the cotyledons, but that a small amount of starch was left in the cotyledons. The supply of soluble carbohydrates in the seedling may have been insufficient to provide for further growth. It has been shown by a number of investigators that leguminous seedlings grown from high-protein seeds of especially high-protein content have insufficient carbohydrate reserves to allow a complete utilization of the stored nitrogen. The possibility of some limiting factor other than a carbohydrate should also be considered. No tests were conducted to determine whether the seedlings could be kept growing for a longer time by immersing the roots in sugar solutions.

Some variations in the relative weights of shoots and roots in relation to differences in temperature have been observed. In one series of experiments with high- and low-protein corn it was noticed that a relatively greater weight of roots was produced at 27° than at 20° C.

Observations made on cross-sections of stems and roots of the dicotyledonous seedlings showed that the processes of both nuclear and cell division have been much limited. Secondary thickening is less advanced than in stems and roots of similar seedlings grown in the light. Some microchemical tests for cell membrane substances have been made. There is some evidence that lignin is present in the xylem vessels of the stem in most of the types of seedlings. Darkness does not prevent the deposition of strengthening materials in the cell walls, although it does limit the process greatly.

II. SEEDLINGS GROWN WITH NITRATES IN NUTRIENT SOLUTION

The quantitative data are presented in table II. When nitrates were obtainable by the growing seedlings, there was found to be a definite increase in the total green weight in thirteen of the nineteen types studied. Two of the types had the same total green weight when nitrates were available as when they were not. A decrease in the total green weight of four types was noted. Possibly in the latter forms carbohydrates were a limiting factor. There is evidence from some of the experiments to be described in a subsequent paper that the presence of nitrates in the nutrient solution stimulates respiration. This alteration in the rate of respiration of seedlings grown in darkness would have considerable effect on the duration of the reserve carbohydrates and fats.

The increases in total green weights of tissues with the use of nitrates are not nearly so great as has been found in the experiments with tomato cuttings, which had a much more abundant store of reserve carbohydrates. It was found that nitrates favored the growth of shoots much more than of roots in the experiments both with seedlings and tomato cuttings. In some of the experiments with cuttings, in which the nitrogen and carbohydrate reserves were both moderately high, the presence of nitrates in the nutrient solution in which the cuttings were grown had a definitely unfavorable effect on the growth of roots. Only four of the nineteen types of seedlings had growth of roots increased by the presence of nitrates in the nutrient solution; thirteen types had root growth somewhat suppressed; and two types had about the same weight of roots with nitrate as without. Growth of shoots was definitely favored in fourteen types of seedlings; it was inhibited in four types; and about the same in two types. These responses as to the stimulating effect of nitrates on growth of shoots also agree with results obtained with tomato cuttings.

SUMMARY OF RESULTS OBTAINED WITH SEEDLINGS GROWN
IN DARKNESS

i. There appears to be a tendency for the relative weights of shoots to roots to vary according to the carbohydrate and nitrogen content of the seed: the higher the supply of carbohydrates in pro-

TABLE II
SEEDLINGS GROWN IN DARKNESS WITH NITRATES AVAILABLE, MARCH 9—APRIL 7, 1925

| PLANT | VARIETY | No. OF PLANTS | LENGTH OF GROWTH PERIOD (DAYS) | WEIGHT OF SEEDS (gm.) | AVERAGE LENGTH OF SHOOTS (cm.) | Roots (cm.) | GREEN WEIGHTS PER PLANT | Ratio OF WEIGHTS OF SHOOTS TO ROOTS (gm.) | Ratio OF WEIGHTS OF SHOOTS TO ROOTS (gm.) | TOTAL GROWTH PER gm. OF RESERVE FOODS (cm.) |
|----------------|-----------------------|---------------|--------------------------------|-----------------------|-----------------------------------|-------------|-------------------------|---|---|---|
| Wheat..... | Blue stem | 40 | 17 | 1.55 | 2.27 | 26.7 | 9.2 | 0.196 | 0.055 | 3.58 |
| Wheat..... | Marquis | 40 | 17 | 1.39 | 2.50 | 28.0 | 9.7 | 0.173 | 0.047 | 3.68 |
| Wheat..... | Little club | 40 | 17 | 1.33 | 1.52 | 24.0 | 9.2 | 0.180 | 0.077 | 2.32 |
| Barley..... | Vermont champion | 20 | 18 | 0.53 | 1.82 | 23.6 | 10.2 | 0.188 | 0.079 | 7.7 |
| Oats..... | Storm King | 31 | 12 | 0.94 | 2.68 | 25.3 | 9.2 | 0.144 | 0.074 | 10.1 |
| Rye..... | Giant winter | 52 | 17 | 1.19 | 1.75 | 22.2 | 10.1 | 0.155 | 0.053 | 7.2 |
| Rice..... | Honduras | 38 | 24 | 0.89 | 1.68 | 30.4 | 9.2 | 0.117 | 0.019 | 2.91 |
| Corn..... | Illinois high-protein | 12 | 22 | 3.27 | 2.76 | 37.1 | 14.8 | 1.340 | 0.427 | 9.1 |
| Corn..... | Illinois low-protein | 12 | 22 | 4.69 | 1.99 | 43.0 | 21.1 | 1.750 | 0.510 | 5.8 |
| Corn..... | Illinois low-oil | 12 | 22 | 7.30 | | 40.5 | 15.2 | 1.480 | 0.377 | 3.0 |
| Peas..... | First of all | 23 | 24 | 4.00 | 4.11 | 53.9 | 8.9 | 1.360 | 0.345 | 9.8 |
| Peas..... | Juno | 23 | 24 | 6.04 | 5.10 | 52.2 | 10.1 | 1.250 | 0.394 | 6.2 |
| Beans..... | White Marrow bush | 9 | 24 | 2.99 | 3.85 | 46.2 | 8.8 | 2.593 | 0.483 | 5.36 |
| Cow peas..... | New era | 20 | 23 | 2.01 | 4.40 | 28.5 | 9.3 | 0.725 | 0.177 | 6.9 |
| Soy beans..... | Peking | 19 | 29 | 1.28 | 3.49 | 46.2 | 5.1 | 0.822 | 0.098 | 8.35 |
| Sunflower..... | Mammoth Russian | 10 | 17 | 0.47 | 4.56 | 29.2 | 8.9 | 1.180 | 0.112 | 27.6 |
| Squash..... | Hubbard | 9 | 29 | 1.31 | 5.10 | 32.6 | 12.2 | 4.793 | 0.676 | 7.08 |
| Melon..... | Red Rocky Ford | 18 | 18 | 0.24 | 5.62 | 13.5 | 3.9 | 0.264 | 0.335 | 22.5 |
| Tomato..... | Bonnie Best Ford | 46 | 18 | 0.12 | ? | 12.7 | 1.8 | 0.061 | 0.004 | 25.0 |

portion to the nitrogen, the lower the relative weights of shoots to roots. The relation is probably not so clear as it might be if the carbohydrate reserve were not a limiting factor to the utilization of nitrogen in some of the high-nitrogen types of seedlings.

2. Nitrate nitrogen is assimilated by seedlings grown in darkness. Variations in ability to synthesize nitrates into growth-promoting substances are doubtless caused to some extent by differences in the carbohydrate or fat reserves, or in some cases by differences in both fats and carbohydrates.

3. Nitrates tend to increase growth of shoots in darkness, but they inhibit growth of roots somewhat, especially if the supply of reserve carbon compounds is much limited.

4. There is a marked inhibition in growth as to size and number of leaves in all types of seedlings investigated except among the representatives of the Gramineae. Observations indicate that this is due partly to a failure of the cells already formed to grow, although there is also a limitation of cell division. In general, the greater compactness of the foliaceous tissues, lack of intercellular spaces, and the small size of cotyledons of the foliaceous type (as in squash) show that growth of cells is inhibited.

5. Growth of roots is much restricted in practically all types of seedlings, but stem elongation is extensive with the exception of that of seedlings of the Gramineae, in which the leaf sheaths are much elongated.

6. The processes of secondary thickening of stems and roots and of deposition of materials in the cell walls are very much inhibited.

Seedlings grown in light in normal atmosphere

Seasonal differences have been found to modify noticeably the growth of seedlings in the light. An attempt has been made to avoid differences caused by changes of season, such as length of day, temperature, and intensity and quality of light. The experiments on the effect of nitrogen starvation, and nitrogen feeding on the growth of different types of seedlings in the light, have been conducted chiefly during the months of October and March. During these months day length is approximately the same, except for the fact that in the October experiments the days became shorter as the experiment progressed, and in March they became longer. How-

ever, since most of the seedlings did not grow for more than three weeks, this difference in length of day at the beginning as compared with the end of the experiment could not have had much influence on the responses.

In the few tests that have been made during the months with longer days, in which the sunlight was also more intense, lower shoot to root ratios were obtained. This is to be noted to some extent by a comparison of the results shown in tables III, IV, VII, and VIII. Although the experiments were carried on during corresponding spring and fall months of the year, in the October experiment the weather was cloudy about half of the time; whereas during the March experiment there was a larger proportion of hours of sunshine. In some experiments conducted during November and the early part of December, it was noted that the shoot to root ratios were considerably higher than in the October experiment and much higher than in the March experiment.

EXPERIMENT I, OCTOBER 1925

Two sets of cultures were prepared in the manner described for seedlings grown in darkness. One set was given the solution lacking nitrates and the other the solution containing nitrates. The experiment was terminated when the seedlings had grown to maximum size, which was determined partially by the time of exhaustion of the food reserves, and partially by observing the time at which the shoots ceased growing. It was found that different kinds of seedlings require different periods of time to reach maximum size. The seedlings of the grass family, especially the low-protein types, attained their development in the shortest time. The cultures receiving nitrates were always harvested on the same day as the corresponding cultures not receiving extra nitrogen.

The temperature of the greenhouse was kept at $21^{\circ}-22^{\circ}$ C. during most of each 24-hour period, but on sunshiny days there was a rise during the middle of the day. All seedlings in each of the two sets of cultures were grown under the same external conditions, so such differences as are noted in the responses must be due to differences in the nature and amount of the food reserves and to the hereditary factors.

1. Seedlings grown without extra nitrogen

Table III gives the quantitative results of this experiment, and figs. 8-13 present illustrations of soy bean, cow pea, muskmelon, sunflower, and low- and high-protein corn seedlings. The ratios of the weights of shoots to roots were much lower than those of corresponding seedlings grown in darkness; but in agreement with the results obtained in darkness, the higher protein types of seeds produced seedlings with higher shoot to root ratios. The low-protein types, such as are found in the Gramineae, developed seedlings with the lowest shoot to root ratios. The high-protein, high-oil seeds yielded seedlings with the highest shoot to root ratios; and, as in the case of the experiments in darkness, seedlings of the Leguminosae were intermediate in their responses.

All seedlings in this experiment had an extensive development of roots in proportion to the size of the shoots. Early in the growth of the seedlings starch began to accumulate in certain tissues. The greatest ability to store starch was noted in tomato, squash, and muskmelon seedlings. It was previously shown by KRAUS and KRAYBILL (8), and later by others, that carbohydrates rapidly accumulate when nitrogen is a limiting factor for protein synthesis and growth. The non-nitrogenous carbon compounds accumulate in sunflower seedlings in the form of oils, although considerable starch is also present. In seedlings of the grass family a relatively large amount of the carbohydrate material appears to be deposited in the cell walls. All tissues of these seedlings undergo rapid differentiation, and the cell walls of the strengthening tissues rapidly increase in thickness. The tissues are physiologically old when the seedlings have grown for only two or three weeks, and the lower the nitrogen content of the seed the more rapid the maturing and eventual senescence of the tissues.

After emerging into the light, the cotyledons and leaves as they grew developed an intense green color. In some cases the color was of a darker and somewhat bluer green than that observed in the plants receiving nitrates. During the latter part of the growth period the color gradually became less intense and developed more of a yellowish tinge. These results as to amount of chlorophyll in leaves and cotyledons of seedlings receiving and not receiving nitrates

TABLE III
SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE WITH NO EXTERNAL SOURCE OF NITROGEN, OCTOBER 7, 1925

| PLANT | VARIETY | No. OF PLANTS | AVERAGE LENGTH OF | | | GREEN WEIGHT PER PLANT | | | RATIO OF WEIGHTS OF SHOOTS TO ROOTS |
|----------------|-----------------------|---------------|--------------------------------|-----------------------------|--------------|------------------------|--------------|--------------|-------------------------------------|
| | | | LENGTH OF GROWTH PERIOD (DAYS) | TOTAL NITROGEN IN SEEDS (%) | Shoots (cm.) | Roots (cm.) | Leaves (cm.) | Shoots (gm.) | |
| Wheat..... | Blue stem | 53 | 14 | 2.27 | 17.0 | 19.2 | 13.2 | 0.146 | 0.198 |
| Wheat..... | Marquis | 53 | 13 | 2.50 | 15.5 | 16.3 | 11.6 | 0.142 | 0.199 |
| Wheat..... | Little club | 56 | 13 | 1.52 | 10.7 | 12.8 | 7.7 | 0.096 | 0.149 |
| Barley..... | Vermont champion | 43 | 14 | 1.82 | 11.2 | 17.0 | 9.4 | 0.158 | 0.198 |
| Barley..... | Storm king | 46 | 18 | 2.68 | 21.0 | 10.9 | 14.5 | 0.171 | 0.268 |
| Oats..... | Clydesdale | 30 | 15 | 2.82 | 11.2 | 10.3 | 7.8 | 0.142 | 0.254 |
| Rye..... | Giant winter | 44 | 15 | 1.75 | 10.5 | 16.5 | 7.8 | 0.086 | 0.091 |
| Rice..... | Honduras | 44 | 22 | 1.68 | 13.2 | 10.5 | 8.6 | 0.056 | 0.069 |
| Corn..... | Illinois high-protein | 10 | 17 | 2.76 | 24.2 | 14.2 | 18.0 | 1.472 | 1.540 |
| Corn..... | Illinois low-protein | 10 | 17 | 1.99 | 14.2 | 11.7 | 9.5 | 0.054 | 0.090 |
| Corn..... | Illinois high-oil | 10 | 19 | | 23.5 | 20.0 | 17.0 | 1.312 | 1.775 |
| Corn..... | Illinois low-oil | 5 | 19 | | 20.5 | 15.2 | 15.2 | 1.060 | 1.932 |
| Peas..... | Juno | 18 | 33 | 5.10 | 17.0 | 12.4 | 2.4 | 1.036 | 1.717 |
| Peas..... | Canada white | 16 | 23 | 3.60 | 23.7 | 12.1 | 2.4 | 0.947 | 0.890 |
| Beans..... | White Marrow bush | 5 | 18 | 3.85 | 15.7 | 11.2 | 6.5 | 3.018 | 1.544 |
| Cow peas..... | New era | 15 | 17 | 4.49 | 8.7 | 12.7 | 5.2 | 1.007 | 0.996 |
| Soy beans..... | Peking | 26 | 21 | 3.40 | 10.1 | 14.7 | 3.4 | 0.533 | 0.376 |
| Sunflower..... | Mammoth Russian | 22 | 25 | 4.56 | 15.0 | 14.0 | 3.9 | 1.250 | 0.757 |
| Squash..... | Hubbard warded | 5 | 33 | 5.10 | 6.3 | 17.0 | 4.5 | 4.144 | 3.032 |
| Melon..... | Red Rocky Ford | 30 | 33 | 5.62 | 6.1 | 6.1 | 1.3 | 0.371 | 2.610 |
| Tomato..... | Bonnie Best | 22 | 21 | | 2.4 | 7.5 | 0.5 | 0.060 | 0.393 |
| | | | | | | | | 0.044 | 1.356 |

agree with those reported by DEUBER (4) for soy bean seedlings. Some of the seedlings had traces of red color in the leaves. Definite changes in pigmentation developed during the growth of the corn seedlings. The leaves of the high-protein corn seedlings had no red color except traces in the midrib at the time of harvest. The low-protein corn seedlings, on the contrary, had much red in the leaves, were much less green, and especially were less bluish-green. The stems of both the high- and low-protein seedlings were very red. Although no red color developed in the wheat seedlings, there were similar differences in the amount and shade of green in the leaves of the high-protein (Marquis) and low-protein (Little Club) types.

2. Seedlings grown with nitrates

The data are presented in table IV. Most of the seedlings began to synthesize nitrates at an early stage of growth. This was especially true of seedlings of the grass family. The leguminous types did not use nitrates rapidly. The composition of the nutrient solution may not have been so well adapted for the growth of seedlings of this family as for the other kinds grown. Also these seedlings may be more sensitive to light conditions than some of the other types, and may require a greater amount of light to enable them to effect a rapid synthesis of nitrates into growth-promoting substances. In some other experiments performed during April and May, when light was of greater intensity and the days were longer, there was a much more rapid synthesis of nitrates by leguminous seedlings as indicated by growth. It may also be possible that, in the sterile cultures, the lack of nodules containing the nitrogen-fixing bacteria may have contributed somewhat in making these seedlings less efficient than the other types in the metabolism of nitrates.

Table V summarizes the effects of the utilization of nitrates on the growth of various organs. There is a striking difference in the effect of nitrates on the growth of shoots and roots. Nitrates increased growth of shoots remarkably, whereas eleven of the nineteen types grown had the total green weight of roots very little affected, and of these eleven types, five varied less than 5 per cent from the weights of roots of seedlings not receiving nitrates, and in four types the roots weighed less when nitrates were present than when they

TABLE IV
SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE WITH NITRATES, OCTOBER 7, 1925

| PLANT | VARIETY | NO. OF PLANTS | LENGTH OF GROWTH PERIOD (DAYS) | AVERAGE LENGTH OF | | | GREEN WEIGHT PER PLANT | | RATIO OF WEIGHTS OF SHOOTS TO ROOTS |
|----------------|-----------------------|---------------|--------------------------------|-------------------|--------------------|--------------|------------------------|-------------|-------------------------------------|
| | | | | Shoots (cm.) | Total in seeds (%) | Leaves (cm.) | Shoots (gm.) | Roots (gm.) | |
| Wheat..... | Blue stem Marquis | 53 | 14 | 2.27 | 23.0 | 15.2 | 19.0 | 0.277 | 0.197 |
| Wheat..... | Little club | 53 | 13 | 2.50 | 22.5 | 15.2 | 18.0 | 0.262 | 0.108 |
| Wheat..... | Vermont champion | 43 | 13 | 1.52 | 19.7 | 14.5 | 13.4 | 0.205 | 0.148 |
| Barley..... | Storm king | 46 | 14 | 1.82 | 22.6 | 14.2 | 17.4 | 0.355 | 0.189 |
| Oats..... | Clydesdale | 30 | 15 | 2.68 | 28.0 | 9.2 | 21.5 | 0.405 | 0.274 |
| Oats..... | Giant winter | 44 | 15 | 2.82 | 19.5 | 11.7 | 14.0 | 0.220 | 0.270 |
| Rye..... | Rye | 44 | 15 | 1.75 | 18.7 | 13.6 | 14.2 | 0.242 | 0.190 |
| Rice..... | Honduras | 22 | 17 | 1.68 | 8.1 | 6.3 | 4.7 | 0.060 | 0.173 |
| Corn..... | Illinois high-protein | 10 | 17 | 2.76 | 32.2 | 16.7 | 24.3 | 0.000 | 1.38 |
| Corn..... | Illinois low-protein | 10 | 17 | 1.09 | 26.8 | 17.0 | 10.4 | 1.468 | 1.472 |
| Corn..... | Illinois high-oil | 19 | 19 | | 31.6 | 16.5 | 19.4 | 1.946 | 1.547 |
| Corn..... | Illinois low-oil | 5 | 19 | | 30.4 | 14.2 | 23.2 | 2.512 | 1.049 |
| Juno..... | Juno | 18 | 33 | 5.10 | 20.0 | 14.2 | 20.0 | 2.559 | 1.507 |
| Peas..... | Canada white | 16 | 23 | 3.60 | 21.6 | 13.2 | 2.5 | 2.776 | 0.987 |
| Peas..... | White Marrow bush | 5 | 18 | 3.85 | 15.2 | 12.0 | 8.0 | 2.730 | 1.018 |
| Beans..... | New era | 17 | 17 | 4.40 | 11.0 | 13.0 | 6.0 | 0.897 | 1.07 |
| Cowpeas..... | Peking | 21 | 3.40 | 11.3 | 11.4 | 10.1 | 3.6 | 1.815 | 1.59 |
| Soy beans..... | Mammoth Russian | 22 | 25 | 4.56 | 24.2 | 19.6 | 7.6 | 4.288 | 1.200 |
| Sunflower..... | Hubbard varietal | 5 | 33 | 5.10 | 13.9 | 10.6 | 10.4 | 12.840 | 3.28 |
| Squash..... | Red Rocky Ford | 30 | 33 | 5.62 | 7.5 | 7.3 | 3.5 | 0.782 | 2.55 |
| Melon..... | Bonnie Best | 22 | 21 | | 2.5 | 7.5 | 5.2 | 0.296 | 0.456 |
| Tomato..... | | | | | | | 0.074 | 0.206 | 4.00 |

TABLE V
EFFECT OF UTILIZATION OF NUTRATES UPON GROWTH OF SEEDLINGS KEPT IN NORMAL ATMOSPHERE

| PLANT | VARIETY | No. OF PLANTS | LENGTH OF GROWTH PERIOD (DAYS) | TOTAL NITROGEN IN SEEDS (%) | TOTAL WEIGHT OF SEEDS (G.M.) | PERCENTAGE INCREASE IN GREEN WEIGHT DUE TO UTILIZATION OF NITRATES | | |
|------------------|-----------------------|---------------|--------------------------------|-----------------------------|------------------------------|--|---------|---------|
| | | | | | | Total | Shoots | Roots |
| Wheat | Blue stem | 53 | 14 | 2.27 | 2.05 | + 42.0 | + 89.0 | + 7.3 |
| Wheat | Marquis | 53 | 13 | 2.50 | 1.84 | + 34.4 | + 83.8 | + 0.8 |
| Wheat | Little club | 56 | 13 | 1.52 | 1.86 | + 63.6 | + 112.9 | + 30.7 |
| Vermont champion | Barley | 43 | 14 | 1.32 | 1.14 | + 60.8 | + 174.9 | + 158.1 |
| Storm king | Barley | 46 | 18 | 2.68 | 1.40 | + 54.5 | + 136.7 | + 4.3 |
| Oats | Clydesdale | 30 | 15 | 2.82 | 1.35 | + 23.7 | + 90.1 | + 2.2 |
| Oats | Giant winter | 44 | 15 | 1.75 | 1.01 | + 59.4 | + 18.0 | + 13.3 |
| Rye | Giant winter | 44 | 22 | 1.68 | 1.04 | + 3.8 | + 18.0 | + 8.5 |
| Rice | Honduras | 10 | 17 | 2.76 | 2.73 | + 48.7 | + 7.7 | + 13.2 |
| Corn | Illinois high-protein | 10 | 17 | 1.09 | 3.91 | + 143.5 | + 266.9 | + 22.8 |
| Corn | Illinois high-oil | 10 | 19 | | 2.73 | + 64.2 | + 66.9 | + 93.4 |
| Corn | Illinois low-oil | 5 | 19 | | 3.04 | + 50.8 | + 73.5 | + 44.1 |
| Peas | Juno | 18 | 33 | 5.10 | 4.73 | + 24.4 | + 40.9 | + 32.2 |
| Peas | Canada white | 16 | 23 | 3.00 | 2.33 | + 22.3 | + 42.3 | + 32.2 |
| Peas | White Marrow bush | 5 | 18 | 3.85 | 1.66 | + 39.8 | + 41.1 | + 37.3 |
| Beans | New era | 15 | 17 | 4.40 | 1.95 | + 32.1 | + 49.3 | + 11.2 |
| Cowpeas | Soy beans | 26 | 21 | 3.40 | 1.75 | + 29.6 | + 35.6 | + 153.0 |
| Mammoth Russian | Sunflower | 22 | 25 | 4.56 | 1.04 | + 204.6 | + 285.4 | + 42.7 |
| Large Hubbard | Squash | 5 | 33 | 5.10 | 0.73 | + 133.7 | + 211.4 | + 333.4 |
| watered | Red Rocky Ford | 30 | 33 | 5.62 | 0.40 | + 61.5 | + 111.0 | + 1.3 |
| Tomato | Bonnie Best | 22 | 21 | | 0.06 | + 255.7 | + 393.3 | + 442.3 |

were not. It is true, however, that the roots of most of the seedlings receiving nitrates had somewhat more branching than those of the plants starved for nitrogen. The roots of most of the nitrated plants were shorter. The strengthening tissues of both roots and stems in most of the seedlings had somewhat less thickening of the cell walls, and the storage carbohydrates were in all cases less abundant than in the plants starved for nitrogen. The total increase due to the use of nitrates was greater for the high-protein, high-oil types of seedlings, but this was partially because these seedlings grew for a longer time. Of the three kinds of wheat grown, the starchy low-protein type (Little Club) made the greatest gain with the use of nitrates; and in the case of corn the starchy low-protein made a greater gain than the less starchy high-protein type.

The influence of light on the growth of different parts of seedlings is shown in table VI.

Effect of light on plants grown without extra nitrogen

The total green weight increased in plants grown without nitrogen, especially in the high-protein forms. The low-protein starchy types gained only slightly. The green weights of stem plus petioles all decreased under the influence of light, and the decreases ranged from 3.1 per cent in the case of stems of cow pea seedlings to 65.1 per cent in the case of stems of soy bean seedlings. There appeared to be no difference between the high- and low-protein types in the responses of stems and petioles to light. In all these experiments the leaf sheaths and petioles have been included with stem tissue, as they were considered to approach more nearly to stem than to leaf tissues in content and composition. The leaves and cotyledons gained in green weight in the light in most cases; however, the leaves of the starchy low-protein seedlings decreased. This loss in weight amounted to 37.8 per cent for Little Club wheat and 41.6 per cent for Illinois low-protein corn. In a general way it may be stated that the higher the nitrogen content of the seed, the greater the increase in green weight of leaves due to the influence of light. The leaves of leguminous seedlings were very responsive, and the growth of leaves of the high-protein, high-oil seedlings was remarkably influenced by light.

TABLE VI
PERCENTAGE INCREASE OR DECREASE IN WEIGHT OF VARIOUS ORGANS DUE TO INFLUENCE OF LIGHT, OCTOBER 1925

| PLANT | VARIETY | PLANTS GROWN WITHOUT NITRATES | | | | | | PLANTS GROWN WITH NITRATES AVAILABLE | | | | | |
|------------------|-----------------------|-------------------------------|--------|------------------|---------------------|---------|---------|--------------------------------------|---------|----------|----------|----------|--|
| | | Total | Shoots | Stems + petioles | Leaves + cotyledons | Roots | Total | Shoots | Roots | Total | Shoots | Roots | |
| Wheat | Blue stem | + 57.1 | - 3.3% | - 32.8% | + 20.2% | + 101.2 | + 95.2 | + 41.3% | + 287.3 | + 51.4 | + 51.4 | + 31.3 | |
| Wheat | Marquis | + 55.0 | - 14.4 | - 51.1 | + 19.8 | + 109.0 | + 109.0 | + 56.4 | + 138.8 | + 155.8 | + 155.8 | + 155.8 | |
| Wheat | Little club | + 9.3 | - 39.2 | - 39.4 | + 37.8 | + 125.8 | + 56.4 | + 13.8 | + 13.8 | + 155.8 | + 155.8 | + 155.8 | |
| Barley | Vermont champion | + 65.5 | - 5.1 | - 53.2 | + 35.6 | + 219.3 | + 103.8 | + 88.8 | + 88.8 | + 225.3 | + 225.3 | + 225.3 | |
| Barley | Storm king | + 80.0 | + 14.0 | - 22.2 | + 47.4 | + 211.6 | + 211.4 | + 181.2 | + 181.2 | + 230.2 | + 230.2 | + 230.2 | |
| Oats | Giant winter | + 7.2 | - 30.9 | - 19.7 | + 75.0 | + 100.5 | + 56.1 | + 56.1 | + 56.1 | + 230.2 | + 230.2 | + 230.2 | |
| Rye | Rye | + 0.8 | - 41.0 | - 56.0 | + 15.0 | + 122.6 | + 49.1 | + 48.7 | + 48.7 | + 215.8 | + 215.8 | + 215.8 | |
| Honduras | Illinois high-protein | + 39.7 | + 0.1 | - 18.9 | + 20.4 | + 124.5 | + 124.5 | + 93.0 | + 93.0 | + 243.8 | + 243.8 | + 243.8 | |
| Corn | Illinois low-protein | - 29.7 | - 50.8 | - 58.3 | + 41.6 | + 6.6 | + 54.6 | + 11.2 | + 11.2 | + 203.3 | + 203.3 | + 203.3 | |
| Corn | Illinois high-oil | + 42.0 | - 6.4 | - 15.1 | + 1.6 | + 129.9 | ... | ... | ... | ... | ... | ... | |
| Corn | Illinois low-oil | + 29.1 | - 11.1 | - 27.9 | + 15.0 | + 105.4 | + 187.9 | + 87.5 | + 87.5 | + 584.8 | + 584.8 | + 584.8 | |
| Peas | Juno | + 103.1 | + 52.6 | ... | ... | + 234.7 | + 176.4 | + 118.4 | + 118.4 | + 360.6 | + 360.6 | + 360.6 | |
| Beans | White Marrow bush | + 81.1 | + 51.4 | - 32.8 | + 148.1 | + 133.5 | + 107.4 | + 64.2 | + 64.2 | + 338.9 | + 338.9 | + 338.9 | |
| Beans | New era | + 101.5 | + 44.9 | - 3.1 | + 624.1 | + 228.3 | + 195.5 | + 128.6 | + 128.6 | + 469.6 | + 469.6 | + 469.6 | |
| Cowpeas | Peking | + 17.8 | - 9.2 | - 65.1 | + 432.6 | + 188.6 | + 34.8 | - 3.7 | - 3.7 | + 558.2 | + 558.2 | + 558.2 | |
| Mammooth Russian | Summer Sunflower | + 78.4 | + 22.5 | - 36.9 | + 268.0 | + 260.9 | + 373.3 | + 373.3 | + 373.3 | + 479.6 | + 479.6 | + 479.6 | |
| Hubbard wanted | Squash | + 116.9 | + 43.8 | - 47.2 | + 40.1 | + 601.8 | + 206.4 | + 167.9 | + 167.9 | + 777.1 | + 777.1 | + 777.1 | |
| Rocky Ford | Melon | + 131.9 | + 47.8 | - 40.2 | + 449.7 | + 656.0 | + 264.2 | + 196.2 | + 196.2 | + 1750.2 | + 1750.2 | + 1750.2 | |
| Bonnie Best | Tomato | + 131.1 | + 50.0 | - 45.0 | + 387.2 | + 780.0 | + 409.2 | + 355.2 | + 355.2 | | | | |

One of the most noticeable effects of light is that of the strongly positive influence which it exerts on the growth of roots. The low-protein starch types had this stimulating effect of light on growth of roots much less than all other kinds of seedlings. The roots of starch low-protein corn seedlings gained 6.6 per cent by the action of light, the roots of rye 75 per cent, and Little Club wheat 125 per cent. Light caused roots of leguminous seedlings to gain from 119 to 282 per cent. The roots of the higher protein seedlings had much greater increases. The gains in weights of roots of high-protein, high-oil seedlings ranged from 602 to 780 per cent.

Effect of light on plants receiving nitrates

The total green weight was increased in the nitrated plants exposed to light as compared with those grown in darkness, with the exception of rice, in which there was no increase either in weight or in size. This feature of the behavior of rice has been pointed out previously. A peculiarity in the growth of the rice seedlings in darkness is that plants receiving and not receiving nitrates both had very much longer shoots than the corresponding plants grown in the light. Light caused an increase in growth of shoots (stems plus leaves) in most types of seedlings, and growth of roots was greatly increased in all of them. The gains in weights of roots ranged from 155 per cent for Little Club wheat to 1750 per cent for tomato seedlings.

EXPERIMENT II, MARCH 5-26, 1926

This experiment was conducted to determine the weekly increments of growth made by various types of seedlings when receiving and not receiving nitrate nitrogen. In the previous experiment it had been found that different seedlings required different lengths of time to use the reserve nitrogen of the seed, and since the seedlings receiving nitrates were harvested at the same time as seedlings starved for nitrogen, it was difficult to compare one type with another because of the different lengths of the period of growth. This experiment was planned to overcome this difficulty. This kind of an experiment, although better for studying the ability of various types of seedlings to utilize nitrates at different stages of germination and early growth, is not so good for comparing the ability of different types to grow

without extra nitrogen. When seedlings of the grass family were two weeks of age at this season of the year many of them had almost ceased growing, although they were still increasing slightly in weight. The higher protein types were still growing by the end of the third week and had not then attained maximum size.

1. Seedlings not receiving nitrates

The quantitative results are given in table VII. Illustrations of tomato and sunflower seedlings are shown in figs. 14, 16, 18, 20, 22, 24, 26, and 28. The weight of shoots in proportion to that of roots tended to decrease week by week. The relative proportion of shoots to roots of seedlings grown from starchy seeds diminished conspicuously during the second week, but there was relatively little change during the third week. The same situation was found with the higher protein seedlings, with the exception of squash, which grew relatively slowly during the first week. As in the previous experiment, the high-protein seedlings had higher shoot to root ratios than the low-protein seedlings. With the exception of the high-protein barley (Dickinson, N.D.), the greatest gains in green weight were made by the roots of all types of seedlings during the second week. Shoots of seedlings of the grass family and of sunflower grew most rapidly during the first week, and those of the leguminous type and squash and tomato during the second week.

2. Seedlings receiving nitrates

The quantitative results are shown in table VIII, and illustrations of tomato and sunflower seedlings in figs. 15, 17, 19, 21, 23, 25, 27, and 29. The shoot to root ratios were higher in the cultures receiving nitrates than in those not receiving them during the entire period of growth, although in several instances the differences at the end of the first week were not appreciable. The ratios of shoots to roots of the nitrated plants tended to become progressively higher week by week, whereas those of the nitrogen-limited plants became progressively lower. With the exception of seedlings of the legumes, there were marked increases in length of shoots and decreases in length of roots with the use of nitrates. Most of the nitrated plants had more branching of the roots, and the leaves were longer and wider.

TABLE VII
SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE AND HARVESTED AT END OF ONE, TWO, AND THREE WEEKS OF GROWTH,
WITHOUT NITRATES, MARCH-APRIL, 1926

| PLANT | VARIETY | No. OF PLANTS | TOTAL NITROGEN IN SEEDS (%) | ONE WEEK | | | TWO WEEKS | | | THREE WEEKS | | | AVERAGE LENGTH AT END OF THIRD WEEK | | | GREEN WEIGHT OF TISSUE PRODUCED AT END OF THIRD WEEK PER 1 GM. OF RESERVE NITROGEN | | |
|--------------------------|--------------|---------------|-----------------------------|-----------------------|------------------------|-----------------------|------------------------|-----------------------|------------------------|------------------------|-----------------------|------------------------|-------------------------------------|------------------------|-----------------------|--|-----------------------|-------|
| | | | | WEIGHT OF SEEDS (gm.) | WEIGHT OF SHOOTS (gm.) | WEIGHT OF ROOTS (gm.) | WEIGHT OF SHOOTS (gm.) | WEIGHT OF ROOTS (gm.) | WEIGHT OF SHOOTS (gm.) | WEIGHT OF SHOOTS (gm.) | WEIGHT OF ROOTS (gm.) | WEIGHT OF SHOOTS (cm.) | WEIGHT OF ROOTS (cm.) | WEIGHT OF SHOOTS (cm.) | WEIGHT OF ROOTS (cm.) | WEIGHT OF SHOOTS (gm.) | WEIGHT OF ROOTS (gm.) | |
| Wheat..... | Marquis | 62 | 2.50 | 2.44 | 0.689 | 0.185 | 0.144 | 0.233 | 0.57 | 0.206 | 0.352 | 0.48 | 1.5 | 3.4 | 14.0 | 12.6 | 56.7 | 35.74 |
| Little club | | 62 | 1.52 | 1.87 | 0.562 | 0.165 | 0.165 | 0.219 | 0.53 | 0.113 | 0.222 | 0.41 | 11.2 | 15.8 | 8.8 | 90.15 | 70.42 | |
| Dats..... | | 62 | 2.62 | 2.78 | 0.602 | 0.170 | 0.188 | 0.244 | 0.59 | 0.167 | 0.292 | 0.56 | 16.4 | 15.8 | 3.6 | 13.2 | 33.46 | |
| H. (Jones and Dickinson) | | 62 | 3.00 | 2.15 | 0.500 | 0.155 | 0.162 | 0.205 | 0.340 | 0.60 | 0.207 | 0.381 | 0.70 | 21.5 | 15.2 | 60.0 | 40.58 | |
| Barley..... | | 62 | 2.70 | 2.58 | 0.505 | 0.155 | 0.156 | 0.205 | 0.345 | 0.53 | 0.207 | 0.376 | 0.53 | 17.6 | 12.6 | 65.0 | 42.38 | |
| Hanichen (Aberdeen) | | 10 | 4.40 | 1.39 | 0.295 | 0.117 | 0.252 | 0.772 | 0.660 | 1.118 | 0.62 | 0.985 | 0.62 | 17.2 | 11.3 | 4.0 | 27.9 | 17.22 |
| Cowpeas..... | | | | | | | | | | | | | | | | 10.60 | 10.60 | |
| Peas..... | First of all | 32 | 4.11 | 5.57 | 0.110 | 0.141 | 0.141 | 0.370 | 0.540 | 1.06 | 1.120 | 1.05 | 20.1 | 13.2 | 2.5 | 16.49 | 16.6 | |
| Peking | | 32 | 3.40 | 2.15 | 0.359 | 0.127 | 0.281 | 0.590 | 0.313 | 1.383 | 1.180 | 1.180 | 1.40 | 11.2 | 12.0 | 3.7 | 47.1 | 37.57 |
| Hubbard | | 14 | 5.10 | 2.04 | 0.693 | 0.338 | 0.265 | 2.369 | 1.103 | 2.483 | 3.759 | 2.361 | 1.45 | 18.5 | 8.5 | 32.88 | 34.75 | |
| Squash..... | | 20 | 4.56 | 0.95 | 0.633 | 0.257 | 0.247 | 0.643 | 0.375 | 1.644 | 1.210 | 0.976 | 1.24 | 13.9 | 4.3 | 55.89 | 43.88 | |
| sunflower..... | | 62 | | 0.16 | 0.030 | 0.013 | 2.26 | 0.070 | 0.016 | 1.54 | 0.073 | 0.073 | 1.02 | 1.1 | 3.4 | 12.5 | 12.1 | |
| Tomato..... | | | | | | | | | | | | | | | | 1.12 | 1.12 | |

TABLE VIII
SEEDLINGS GROWN IN LIGHT IN NORMAL ATMOSPHERE AND HARVESTED AT END OF ONE, TWO, AND THREE WEEKS OF GROWTH,
WITH NITRATES, MARCH-APRIL, 1926

| PLANT | VARIETY | No. OF PLANTS | TOTAL NITROGEN IN SEEDS (%) | WEIGHT OF SEEDS (gm.) | ONE WEEK | | TWO WEEKS | | THREE WEEKS | | AVERAGE LENGTH AT END OF THIRD WEEK | | | | GREEN WEIGHT OF TISSUE PRODUCED AT END OF THIRD WEEK PER 0.1 GM. OF RESERVE NITROGEN | | | | |
|------------------|----------------------|---------------|-----------------------------|-----------------------|------------------------|---------------------------------|------------------------|---------------------------------|------------------------|---------------------------------|-------------------------------------|--------------|-------------|--------------|--|-------------|--------------|--------------|-------------|
| | | | | | Weight of shoots (gm.) | Weight of roots (gm.) to shoots | Weight of shoots (gm.) | Weight of roots (gm.) to shoots | Weight of shoots (gm.) | Weight of roots (gm.) to shoots | Shoots (cm.) | Leaves (cm.) | Total (cm.) | Shoots (gm.) | Leaves (gm.) | Total (gm.) | Shoots (gm.) | Leaves (gm.) | Total (gm.) |
| Wheat..... | Marquis | 62 | 2.50 | 2.44 | 0.159 | 0.113 | 1.32 | 0.468 | 0.279 | 1.46 | 0.893 | 0.328 | 2.72 | 36.6 | 8.3 | 124.1 | 90.81 | 33.3 | |
| Wheat..... | Little club | 62 | 1.52 | 1.87 | 0.097 | 0.115 | 0.84 | 0.277 | 0.239 | 1.15 | 0.658 | 0.331 | 2.01 | 31.6 | 11.2 | 25.2 | 145.8 | 72.3 | |
| Oats..... | Clydesdale | 62 | 2.82 | 2.78 | 0.149 | 0.150 | 0.90 | 0.394 | 0.269 | 1.47 | 0.871 | 0.318 | 2.73 | 42.9 | 13.9 | 92.1 | 165.9 | 25.2 | |
| Barley..... | Hannchen (Dickerson) | 62 | 2.76 | 2.11 | 0.210 | 0.212 | 0.99 | 0.365 | 0.362 | 1.83 | 1.120 | 0.447 | 2.51 | 37.9 | 10.7 | 30.4 | 166.9 | 47.6 | |
| Barley..... | Hannchen (Aberdeen) | 62 | 2.20 | 2.50 | 0.233 | 0.261 | 0.89 | 0.731 | 0.379 | 1.92 | 1.110 | 0.433 | 2.57 | 37.8 | 9.2 | 30.3 | 173.9 | 48.8 | |
| Cowpeas..... | New era | 10 | 4.40 | 1.30 | 0.332 | 0.130 | 2.55 | 1.339 | 0.804 | 1.66 | 1.300 | 1.000 | 1.37 | 5.0 | 5.0 | 4.9 | 24.3 | 17.6 | |
| Peas..... | First of all | 32 | 4.11 | 5.57 | 0.123 | 0.134 | 0.92 | 0.915 | 0.656 | 1.39 | 1.553 | 0.681 | 1.98 | 29.1 | 5.0 | 2.5 | 38.4 | 18.9 | |
| Soy bean..... | Peking | 32 | 3.40 | 2.15 | 0.300 | 0.106 | 3.41 | 0.702 | 0.360 | 2.33 | 0.880 | 0.518 | 1.79 | 13.0 | 10.0 | 4.4 | 38.5 | 22.7 | |
| Squash..... | Hubbard | 14 | 5.10 | 2.04 | 1.579 | 0.468 | 3.37 | 6.107 | 1.736 | 3.52 | 1.4500 | 3.403 | 4.26 | 35.4 | 17.0 | 10.0 | 241.0 | 105.2 | |
| Mammooth Russian | 20 | 4.56 | 0.95 | 0.714 | 0.245 | 2.91 | 2.011 | 0.011 | 3.29 | 4.660 | 0.902 | 5.16 | 32.8 | 11.3 | 8.2 | 72.2 | 55.6 | 16.5 | |
| Sunflower..... | Tomato..... | 62 | | 0.16 | 0.067 | 0.015 | 4.40 | 0.434 | 0.061 | 7.15 | 1.135 | 0.134 | 8.46 | 20.2 | 8.1 | 7.5 | 256.8 | 215.2 | 41.6 |

Both shoots and roots of all the types of seedlings made the greatest gains in green weight during the second week. At the end of the first week the nitrated seedlings of the grass family had greener leaves than the seedlings not receiving nitrogen. Oat seedlings were an exception in this respect. There was no difference in the greenness of the two sets of leaves in the three types of leguminous seedlings. This condition was correlated with little difference in size of the nitrated and un-nitrated cultures. There was very little difference in color of the cotyledons of tomato, squash, and sunflower seedlings of the two sets of cultures at the end of the first week.

At the end of the second week the first leaves of seedlings of the grass family which were not receiving nitrates were beginning to die at the tips, and the general color of the leaves was a yellower green than that of the nitrated seedlings. With the exception of cow pea seedlings, in which there was no difference in color, the leguminous seedlings not receiving nitrates had greener leaves at the end of the second week. The cotyledons and leaves of sunflower and squash and the cotyledons of tomato seedlings not receiving nitrates were greener than those of plants receiving nitrates.

At the end of the third week all plants receiving nitrates had greener leaves than those of the nitrogen-starved plants.

It has been found in both the March (1925) and October (1926) experiments that seedlings of the grass family developed signs of starvation for nitrogen earlier than the higher protein types. Seedlings from the latter type of seeds had the capacity to continue growth for a longer time. There also appeared to be a difference in the amount of green tissue produced per 0.1 gm. of reserve nitrogen. The results of the October experiment are given in table IX, and of the March experiment in tables X and XI. The four types of high-protein, high-oil seedlings differ from all the others in this respect. Per unit of reserve nitrogen their total green weight is greater, and the difference in weight as compared with seedlings grown from the low-protein seeds is brought about by a greater growth of stems and leaves. There is no distinct difference in the weight of roots produced per unit of nitrogen by the different classes of seedlings. Just what is the cause or significance of this increased growth by the high-protein, high-oil seedlings cannot be stated. The high-protein, high-oil types

TABLE IX
GREEN WEIGHT OF TISSUE IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS GROWN WITH NO EXTERNAL
SOURCE OF NITROGEN, MARCH-APRIL, 1924

| PLANT | VARIETY | AMOUNT OF N PER SEED (MG.) | DARKNESS | | | LIGHT | | |
|-----------------|-----------------------|----------------------------|----------|-------------------------|---------------------|-------|-------------------------|---------------------|
| | | | Total | Shoots (stems + leaves) | Leaves + cotyledons | Total | Shoots (stems + leaves) | Leaves + cotyledons |
| Wheat..... | Blue stem | 0.878 | 24.9 | 17.2 | 7.8 | 9.4 | 7.7 | 35.4 |
| | Marquis | 0.982 | 22.4 | 16.9 | 8.2 | 8.7 | 5.5 | 32.4 |
| Wheat..... | Little club | 0.504 | 44.4 | 31.3 | 14.1 | 17.2 | 13.1 | 48.6 |
| | Vermont champion | 0.482 | 49.9 | 28.0 | 12.9 | 15.1 | 12.8 | 62.0 |
| Barley..... | Storm king | 0.817 | 28.8 | 18.3 | 8.8 | 9.5 | 10.5 | 49.1 |
| Oats..... | Giant winter | 0.401 | 41.1 | 28.1 | 10.4 | 17.7 | 12.9 | 61.9 |
| Rye..... | Rice..... | 0.396 | 31.7 | 24.0 | 19.0 | 5.0 | 7.8 | 19.0 |
| Corn..... | Illinois high-protein | 7.53 | 28.6 | 19.5 | 10.3 | 9.2 | 9.1 | 29.0 |
| | Illinois low-protein | 4.26 | 47.8 | 30.2 | 16.7 | 13.5 | 17.6 | 33.7 |
| Peas..... | Hano | 13.4 | 13.3 | 9.4 | 8.5 | 0.9 | 3.8 | 13.6 |
| Beans..... | White Marrow bush | 12.8 | 19.7 | 15.5 | 14.6 | 0.9 | 4.1 | 32.3 |
| Cow peas..... | New era | 5.74 | 17.2 | 13.0 | 12.9 | 0.1 | 4.1 | 26.8 |
| Soy beans..... | Peking | 2.29 | 35.5 | 28.0 | 25.1 | 2.9 | 7.5 | 39.6 |
| Mammoth Russian | 2.17 | 51.8 | 47.0 | 39.4 | 7.6 | 4.8 | 80.3 | 92.5 |
| Squash..... | Hubbard | 9.83 | 33.5 | 29.1 | 23.8 | 5.3 | 4.4 | 67.9 |
| Melon..... | Red Rocky Ford | 0.753 | 38.6 | 33.3 | 28.0 | 5.3 | 83.4 | 45.9 |
| Tomato..... | Bonnie Best | 0.117 | 38.4 | 34.2 | 27.6 | 6.6 | 4.2 | 80.0 |

TABLE X
GREEN WEIGHT OF TISSUES IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS HAVING NO EXTERNAL SOURCE OF NITROGEN, MARCH 5-26, 1926

| PLANT | VARIETY | ONE WEEK | | | | | | TWO WEEKS | | | | | | THREE WEEKS | | | | | | |
|------------------|----------------------|---------------|--------|-----------------------------|--------|-----------------------|-------|-----------|-------|--------|-------|--------|-------|-------------|-------|--------|-------|--------------------------|-------|-------|
| | | NO. OF PLANTS | | TOTAL NITROGEN IN SEEDS (%) | | WEIGHT OF SEEDS (GM.) | | SHOOTS | | ROOTS | | TOTAL | | SHOOTS | | ROOTS | | TOTAL | | |
| | | Shoots | Roots | Shoots | Roots | Shoots | Roots | Shoots | Roots | Shoots | Roots | Shoots | Roots | Shoots | Roots | Shoots | Roots | Shoots | Roots | |
| Wheat..... | Marquis | 62 | 2.50 | 2.44 | 20.50 | 9.83 | 10.72 | 37.40 | 13.67 | 23.70 | 56.70 | 20.95 | 35.74 | 10.66 | 15.66 | 10.49 | 15.66 | 10.49 | 15.66 | |
| Wheat..... | Little club | 62 | 1.52 | 1.87 | 32.40 | 12.17 | 20.22 | 59.82 | 16.80 | 43.07 | 89.70 | 16.24 | 33.38 | 13.20 | 27.57 | 13.71 | 47.2 | 19.69 | 50.48 | 34.75 |
| Wheat..... | Clydesdale | 62 | 2.82 | 2.78 | 16.30 | 7.67 | 16.48 | 26.32 | 8.70 | 11.40 | 19.28 | 36.61 | 13.20 | 40.58 | 16.24 | 36.22 | 69.00 | 28.45 | 40.58 | 45.08 |
| Oats..... | Hannchen (Dickinson) | 62 | 2.76 | 2.11 | 42.80 | 16.30 | 8.67 | 58.10 | 10.10 | 21.84 | 36.22 | 19.28 | 36.61 | 13.20 | 40.58 | 16.24 | 36.22 | 69.00 | 28.45 | 40.58 |
| Barley..... | Hannchen (Aberdeen) | 62 | 2.20 | 2.50 | 33.10 | 15.56 | 17.58 | 59.70 | 20.85 | 38.89 | 65.00 | 22.65 | 42.38 | 13.49 | 11.54 | 25.00 | 17.90 | 5 leaves had dropped off | 17.22 | 62.1 |
| Barley..... | New era | 10 | 4.40 | 1.30 | 7.20 | 5.16 | 1.30 | 7.20 | 5.16 | 1.30 | 7.20 | 5.16 | 1.30 | 7.20 | 5.16 | 1.30 | 7.20 | 5.16 | 1.30 | 7.20 |
| Cow peas..... | | | | | | | | | | | | | | | | | | | | |
| First of all | Peas..... | 32 | 4.11 | 5.57 | 3.50 | 1.54 | 1.96 | 15.5 | 7.97 | 7.55 | 32.1 | 10.49 | 15.66 | 10.49 | 15.66 | 10.49 | 15.66 | 10.49 | 15.66 | 10.49 |
| Peking | Soy bean..... | 32 | 3.40 | 5.15 | 21.3 | 15.7 | 5.58 | 39.5 | 25.83 | 13.71 | 47.2 | 27.57 | 13.71 | 47.2 | 13.71 | 47.2 | 27.57 | 13.71 | 47.2 | 13.71 |
| Hubbard | Squash..... | 14 | 5.10 | 2.04 | 13.9 | 9.33 | 4.55 | 54.6 | 38.94 | 15.65 | 85.2 | 50.48 | 15.65 | 85.2 | 15.65 | 85.2 | 50.48 | 15.65 | 85.2 | 15.65 |
| Mammooth Russian | Sunflower..... | 20 | 4.56 | 0.95 | 41.2 | 29.33 | 11.87 | 70.1 | 43.57 | 26.56 | 100.9 | 55.89 | 26.56 | 100.9 | 26.56 | 100.9 | 55.89 | 26.56 | 100.9 | 55.89 |
| Bonnie Best | Tomato..... | 62 | 0.0729 | 0.0729 | 62 gm. | 36.8 | 25.6 | 11.2 | 99.0 | 59.5 | 39.5 | 125.6 | 63.5 | 39.5 | 125.6 | 63.5 | 39.5 | 125.6 | 63.5 | 39.5 |

TABLE XI
GREEN WEIGHT OF TISSUES IN GRAMS PRODUCED PER 0.1 GM. OF RESERVE NITROGEN BY SEEDLINGS RECEIVING NITRATES,
MARCH 5-26, 1926

of seedlings also have much the greatest efficiency in the metabolism of inorganic nitrogen.

Discussion

The questions raised in the introduction will now be considered.

1. What are the responses of seedlings having different amounts of reserve carbon and nitrogen when extra amounts of carbon and nitrogen are supplied externally?

The relations of varying amounts of available carbon and nitrogen to the growth of shoots and roots of seedlings are much like those found with tomato cuttings. Seedlings grown from seeds having large carbon reserves in proportion to the nitrogen tend to have low shoot to root ratios when grown without an external source of nitrogen, and those grown from seeds having relatively large amounts of nitrogen have higher shoot to root ratios if the seedlings are grown in the light. If the seedlings are grown in darkness there appears to be a somewhat similar, although considerably less definite, relation between the type of growth and the kind and amount of the food reserves.

The quantitative differences in the growth of different organs of seedlings kept in the light appear to be more directly related to the amounts of available carbon and nitrogen than to genetical affiliations. Within the grass family, for example, we find variations in growth related to the amount of stored foods. The very starchy, low-protein types of wheat, barley, and corn have lower shoot to root ratios than the corresponding somewhat less starchy and higher protein types. The results obtained with the four kinds of seedlings grown from high-protein, high-oil seeds, representing three different families, also indicate that the responses are related to the types and quantities of reserve foods.

When extra nitrogen is supplied, growth is in most cases slightly increased in darkness and is greatly increased in the light. The extra nitrogen favors growth of shoots more than that of roots. The roots of the nitrated seedlings are shorter. In no case was the increase with the use of nitrates in darkness equal to that obtained with the very high-carbohydrate tomato cuttings. It is supposed that this quantitative difference in the responses of seedlings as

compared with the cuttings resulted from the smaller amount of carbohydrate in proportion to the stored nitrogen in the seeds. Tomato stem cuttings may be produced which contain thirty-six parts of carbohydrates (starch, sugar, free-reducing substances) to one of nitrogen; whereas the starchy wheat grains have only about seven parts of carbohydrates to one of nitrogen. The carbohydrates may thus tend to become limiting factors in the growth of seedlings in darkness more quickly than they did in the growth of cuttings.

2. Is fertilization with nitrogen advisable in the early growth of all types of seedlings, regardless of weather and light conditions and the nitrogen reserves of the seed?

The results obtained in some experiments with seedlings grown at different seasons of the year show that, with the exception of rice seedlings, all the types can assimilate nitrate nitrogen into growth-promoting substances in the early phases of growth, if light conditions are favorable and permit of rapid and abundant synthesis of carbohydrates. On the other hand, if conditions are unfavorable for the synthesis of carbohydrates, the very high-nitrogen types of seedlings may grow as well without as with nitrates in their early growth. Nitrates become beneficial after the seedlings have developed a photosynthesizing surface. The rate of nitrogen assimilation is closely correlated with the rate of synthesis of carbon compounds. This sort of response also agrees to some extent with results found with tomato cuttings. There it was noted that nitrates were toxic to cuttings having a very high-nitrogen but low-carbohydrate content when kept in darkness, and that nitrates were of no directly noticeable benefit in the light until more carbohydrates had accumulated by photosynthesis.

3. Is the seedling with the larger reserve of carbon better able to live and thrive during prolonged periods of dull, cloudy weather during the early growth of the plant?

4. Is the seedling with the larger amount of stored nitrogen at its disposal better able to withstand conditions of drought or of poor soil during the early phase of development when nitrogen from an outside source is unavailable or available in very limited amounts?

There is some evidence that those seedlings endowed by the parent plant with an abundance of readily available carbon com-

pounds will make the most rapid growth at first. This is true, not only of those with a limited nitrogen supply, but also of those grown from higher-nitrogen, high-fat seeds in which there is a great abundance of sugar produced by a rapid hydrolysis of the fats. Sunflower and tomato seedlings have this mode of behavior. These two types of seedlings and those of the grass family grew very rapidly during the first week. The results suggest that seedlings having an abundance of readily obtainable carbon reserves are better able to grow during cloudy weather in the early stages of growth. On the other hand, the seedlings having the larger stores of nitrogen are better able to grow and establish themselves in nitrogen-poor soil. They will tend to become deep-rooted under such conditions, and should be able to absorb a greater amount of nitrogen from the substrate because of the wider spread of roots. A period of abundant nitrogen and relatively small carbon supply in the early stages of growth tends to make leafy but shallow-rooted plants, which may not be so well adapted to obtain their needed amount of nitrogen and other minerals in their later development.

KOSINSKI (7), who was probably the first to study this relation of nitrogen to the growth of different organs, found that nitrogen feeding restricts the growth of roots in length and favors that of the stem. GODLEWSKI, in whose laboratory part of KOSINSKI's experiments were conducted (the work was begun in the botanical laboratory at Jena), incidentally utilized some of his barley seedlings for proof of KOSINSKI's results. His observations (6) agreed with those of KOSINSKI, and showed besides that the presence of sugar favored the growth of roots in length. He states that:

root growth was especially favored in comparison with shoot growth if the nitrogen-free solution contained sugar, probably because in the more abundant supply of carbohydrate material, the nitrogen-hunger was accentuated. It seems as if the plants, hungering for nitrogen, strive through the appropriation of the largest possible quantity of the materials available for growth of roots to make better use of the scanty nitrogen supply in the soil.

In the same manner as GODLEWSKI has attempted to account for the favoring of growth of roots by a limited supply of nitrogen and an abundance of carbohydrates, one might explain the stimulation in growth of shoots (particularly of leaves that results when

nitrogen is abundant) as an effort on the part of the plant to expose a great amount of surface to the light so as better to provide for an increase in the products of photosynthesis. It seems more probable, however, that the differences in growth are due more directly to differences in the chemical conditions within the tissues rather than that they develop as purposeful adaptations.

GODLEWSKI (5) observed that:

the roots of etiolated plants are usually shorter than those of plants grown in the light, yet the difference is so small that on no account can the greater length of the hypocotyl of (plants grown in darkness) be compensated by the decrease in length of the roots.

The effect of light on the growth of roots has been neglected by most of the investigators who have studied the characteristics of the etiolated plant in contrast with those of the plant grown in the light. Perhaps the most conspicuous feature of the results reported here is that of the stimulating effect of light on root development. The light-favoring effect on growth of roots is most pronounced in the case of seedlings grown from high-protein seeds. Some of the low-protein types, as low-protein corn, produce root systems as large in darkness as in light when nitrogen is lacking in the nutrient medium. A similar response was noted with tomato cuttings. If they had a very large supply of carbohydrates and were not given extra nitrogen, roots grew almost as well in darkness as in light. It thus seems probable that the light-favoring effect on the growth of roots is directly or indirectly connected with the synthesis of carbohydrates.

These experiments have also demonstrated the well known fact that light has a limiting influence on growth in length of stems. Whether the growth-inhibiting effect is due indirectly to the synthesis and accumulation of carbohydrates, or to its effect on nitrogen and other forms of mineral metabolism, or to more directly stimulating effects on the protoplasm itself, has long been a much discussed question, and one for which a definite answer is still lacking. If seedlings having a supply of reserve carbohydrates are grown in light in an atmosphere lacking carbon dioxide, growth of the stem may equal that of a plant kept in the normal atmosphere under conditions that permit the rapid synthesis of carbohydrates. From this it has been concluded that the growth-limiting effects of light

on the stem cannot be directly due to the synthesis of carbohydrates. GODLEWSKI's (5) experiments have shown that a greater quantity of dry matter may pass from the cotyledons into the hypocotyl, if seedlings are grown in darkness than if grown in light in an atmosphere lacking carbon dioxide. He found that stems of 14-day old *Phaseolus* seedlings grown from seeds of the same weight contained 202 mg. of dry matter when grown in darkness, and 141 mg. of dry matter when grown in light in air lacking CO₂. It may be concluded that the hypocotyls of etiolated plants are longer than those of plants grown in the light, partly because more of the food reserves are used in their growth, and also because they have a considerably higher water content.

GODLEWSKI (5) also sought to determine whether the growth-limiting effects of light on the hypocotyl were exerted directly on it, or more indirectly through its influence in favoring the growth of the cotyledons. Using black paper cases, he darkened the cotyledons only of some etiolated *Raphanus* seedlings and the hypocotyls only in others. Similar seedlings were left uncovered and set out in normal atmosphere. He found that darkening the hypocotyl only caused a definite increase in its length; secondary to this, the cotyledons of the plants with the darkened hypocotyls were somewhat smaller than those of plants grown wholly in the light. Darkening the cotyledons only had a strong influence on their growth; they were larger than the cotyledons of completely etiolated plants. He stated, however, that the cotyledons became somewhat green at the base. It may be possible that the traces of light, which undoubtedly must have entered, caused a stimulation in growth of the cotyledons. BATALIN (1) presented some experimental evidence which showed that illumination of short duration exerts a remarkable influence on the growth of leaves. GODLEWSKI also has shown that illumination for a brief period favors the growth of cotyledons of *Raphanus* seedlings. Later experiments of TRUMPF (15) have demonstrated similar results.

The effects of light on the growth and development of different organs of seedlings will be discussed more fully in two following papers. Additional data will also be presented which help somewhat to indicate to what extent the influence of light on the growth of

different organs is due to the processes of assimilation and utilization of carbohydrates, and to what extent to the influence of light on metabolism and growth independently of carbohydrate synthesis.

SUMMARY OF RESULTS OBTAINED WITH SEEDLINGS GROWN IN LIGHT

1. Seedlings developed from seeds with high-nitrogen content, when grown with no external source of nitrogen, have a greater weight of shoots in proportion to that of roots than those which develop from seeds with low-nitrogen content.
2. The weights of shoots in proportion to roots of seedlings grown in the light is lower than those of seedlings grown in darkness. The shift in the proportions is brought about especially by the shorter stems, and in most cases by much larger root systems of the seedlings grown in the light.
3. The difference in the effect of light and darkness upon the weight of tops in proportion to roots is greater with seedlings from high-protein seeds grown without extra nitrogen than with seedlings grown from low-protein seeds.
4. Light does not greatly favor growth of seedlings from low-protein starchy seeds unless extra nitrogen is supplied. The leaves of these plants are considerably smaller and even weigh less in the light. The roots of some of these seedlings gain considerably in the light, however.
5. Light favors the assimilation of nitrates, especially by high-protein seedlings with relatively low carbon reserves. The assimilation of nitrates and utilization of the synthesized products favor the growth of shoots more than of roots.
6. When the nitrogen supply is limited to the reserves in the seed, the shoot to root ratios tend to diminish after the first week, but if nitrogen is abundantly supplied, the ratio tends to increase continuously during the first three weeks of growth of seedlings in the light.
7. The relative proportions of shoots to roots vary with the season of the year. In the long-day months the ratio is relatively low and in the short-day months it is higher.
8. Light favors the process of secondary thickening in the roots

and stems of dicotyledons, and of thickening of cell walls in all types of seedlings.

General summary

1. Growth of the seedling is influenced by the nature and relative amounts of the food reserves of the seed, as well as by differences in the external environment such as light and darkness, and the presence and absence of nitrates in the nutrient solution. When the seedlings are grown without nitrogen from an outside source the following responses have been found: (a) Seeds having a high-nitrogen and relatively low-carbon content produce seedlings with a large top in proportion to the roots. (b) Seeds having a low-nitrogen and high-carbon content produce seedlings with a relatively small top in proportion to the size and weight of the roots. (c) Seeds intermediate in the proportions of their reserves of carbon and nitrogen produce seedlings with intermediate proportions of shoots to roots.

The following table illustrates the nature of the results:

TABLE XII
WEIGHT IN GRAMS OF SHOOTS AND ROOTS PER PLANT

| RESERVE FOODS | LIGHT | | | DARKNESS | | |
|--------------------------|------------------|--------|-------|----------|--------|-------|
| | Stem petioles | Leaves | Roots | Stems | Leaves | Roots |
| | | | | | | |
| Moderately high nitrogen | | | | | | |
| High-protein corn . . . | 0.634 | 0.838 | 1.540 | 0.774 | 0.696 | 0.686 |
| Moderately high starch | | | | | | |
| Low nitrogen | | | | | | |
| Low-protein corn . . . | 0.298 | 0.336 | 0.800 | 0.715 | 0.575 | 0.750 |
| High starch | | | | | | |
| Moderately high nitrogen | | | | | | |
| White Marrow beans | 1.264 | 1.754 | 1.544 | 1.882 | 0.111 | 0.526 |
| Moderately high starch | | | | | | |
| High nitrogen | | | | | | |
| Cow peas | 0.677 | 0.420 | 0.906 | 0.699 | 0.058 | 0.237 |
| Moderately low starch | | | | | | |
| High nitrogen | | | | | | |
| Sunflower | 0.643 | 0.607 | 0.757 | 0.855 | 0.165 | 0.105 |
| High oil | | | | | | |
| Muskmelon | 0.150 | 0.221 | 0.303 | 0.211 | 0.040 | 0.040 |
| Very high nitrogen | | | | | | |
| High oil | | | | | | |

2. Nitrates are synthesized into growth-promoting substances, both in light and in darkness, but much more rapidly in the light.

3. Nitrates favor the growth of shoots more than of roots.
4. Light strongly favors the growth of roots.
5. (a) Seedlings developed from high-protein seeds benefit most under the influence of light. The roots and leaves are larger, more numerous, and much heavier than in the case of seedlings grown in darkness. This applies to high-protein seedlings grown with and without extra nitrogen, but the effect is greater in the case of the latter. (b) Seedlings grown from low-protein seeds without extra nitrogen are influenced less by light as to weight of different organs. Leaves of the very low-protein types grow even less in light than in darkness. When extra nitrogen is supplied these seedlings also benefit by the influence of light.
6. Seedlings with limited nitrogen supply undergo rapid differentiation and maturing of tissues in the light. The lower the nitrogen content of the seed the more rapid the process.
7. Light favors secondary thickening in stems and roots and deposition of strengthening materials in the cell walls.
8. The responses as to the effect of varying amounts of reserve carbon and nitrogen on growth of the seedling agree with results obtained with tomato cuttings having similar (although in some cases more extreme) variations in composition of the reserves.

These investigations were conducted at Boyce Thompson Institute for Plant Research, in 1924-1926. I wish to express my appreciation to the Cereals Division of the United States Department of Agriculture for the samples of pure line Marquis wheat and Hännchen barley used in these experiments; also to the Department of Plant Genetics of the University of Illinois for the seeds of high- and low-protein corn.

STERLING HALL OF MEDICINE
DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
YALE UNIVERSITY

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EXPLANATION OF PLATES I-IV

PLATE I

Seedlings grown in darkness without nitrates:

- FIG. 1.—Soy bean.
- FIG. 2.—Cow pea.
- FIG. 3.—Muskmelon.
- FIG. 4.—Tomato.
- FIG. 5.—Sunflower.
- FIG. 6.—Illinois low-protein corn.
- FIG. 7.—Illinois high-protein corn.

PLATE II

Seedlings grown in light without nitrates:

- FIG. 8.—Soy bean.
- FIG. 9.—Cow pea.
- FIG. 10.—Muskmelon.

FIG. 11.—Sunflower.

FIG. 12.—Illinois low-protein corn.

FIG. 13.—Illinois high-protein corn.

PLATE III

Tomato seedlings grown in light:

FIGS. 14, 16, 18, and 20.—In nutrient medium lacking nitrogen at stages of growth at one, two, three, and four weeks respectively.

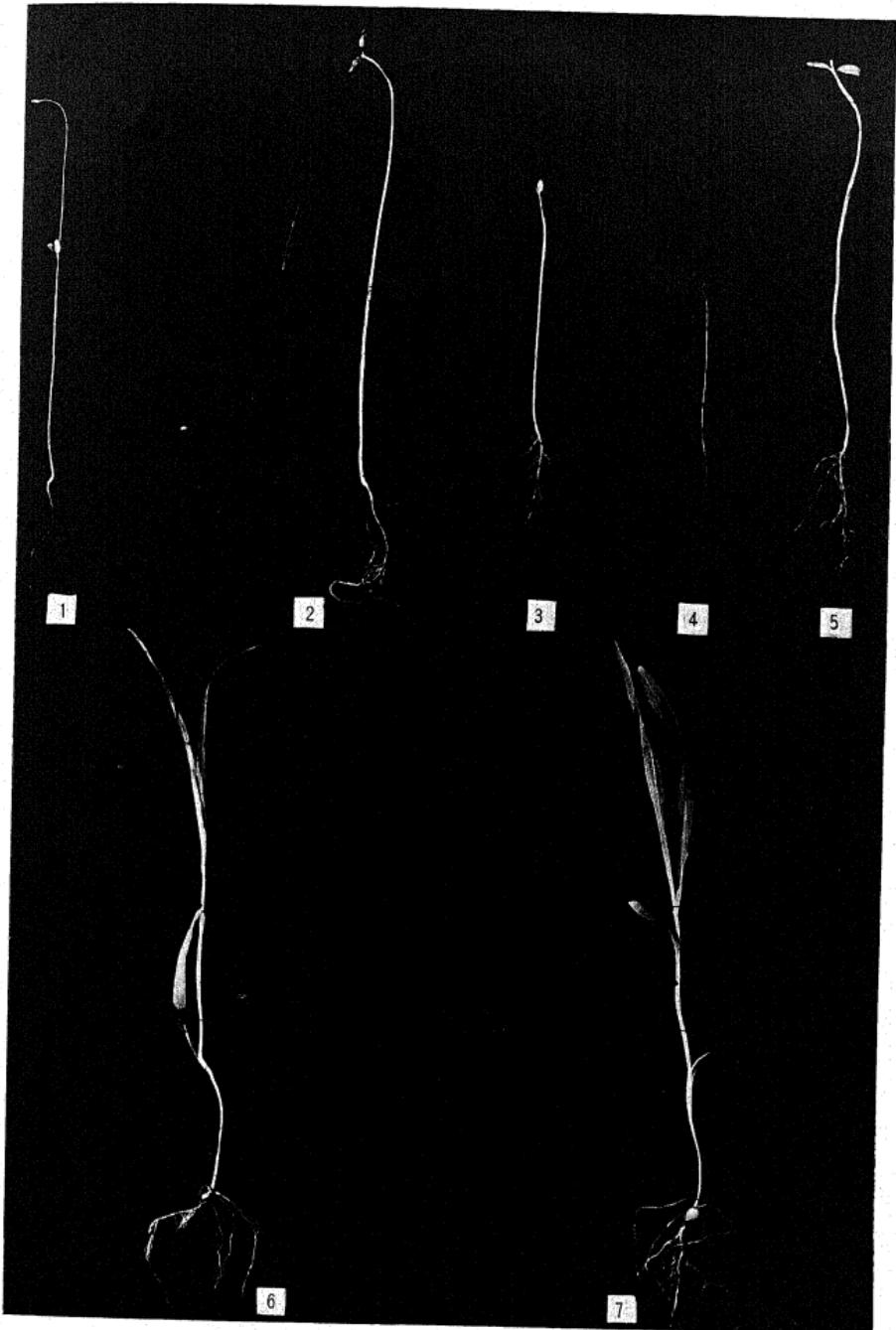
FIGS. 15, 17, 19, and 21.—In nutrient medium containing nitrates at stages of growth of one, two, three, and four weeks respectively.

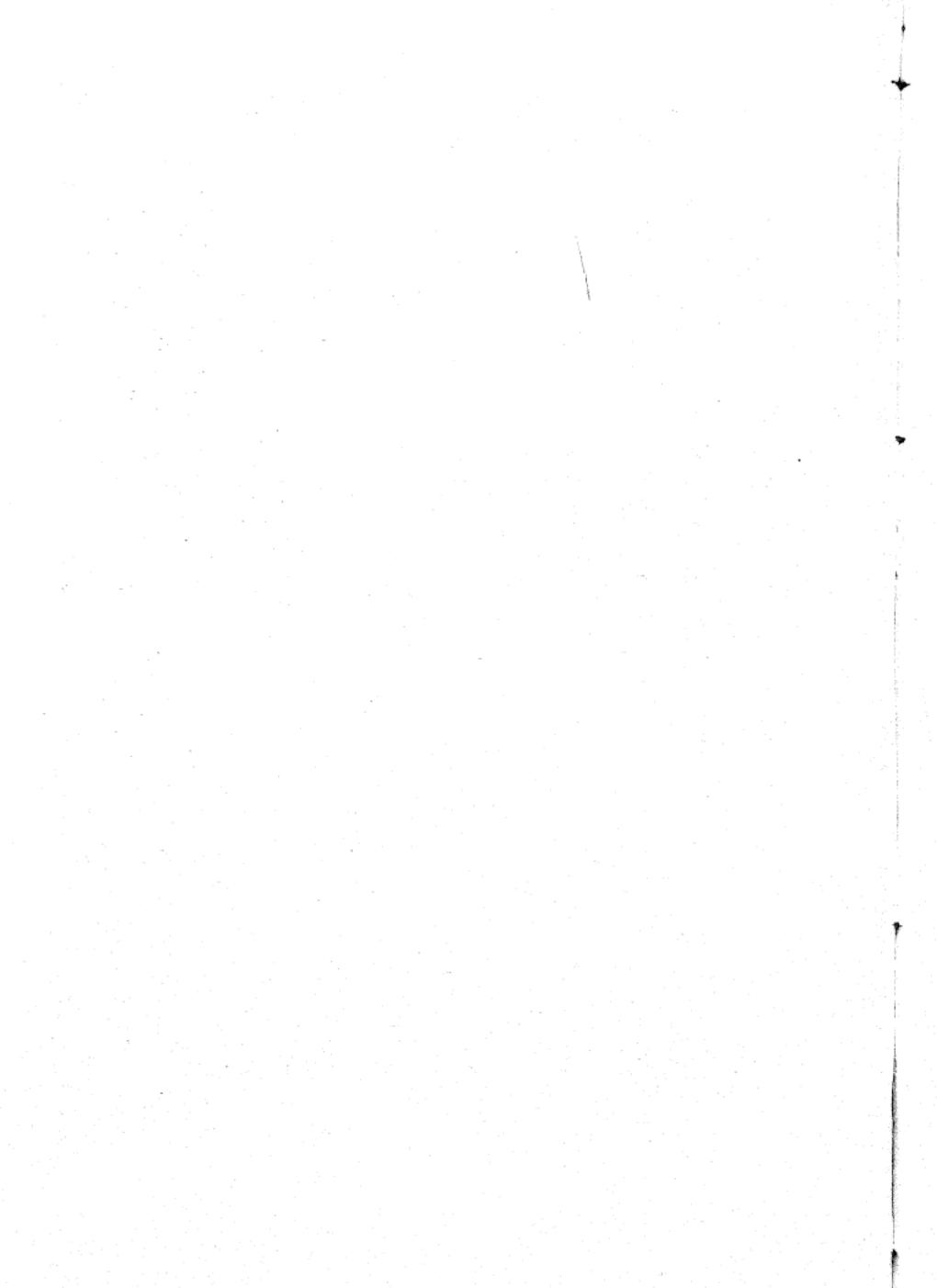
PLATE IV

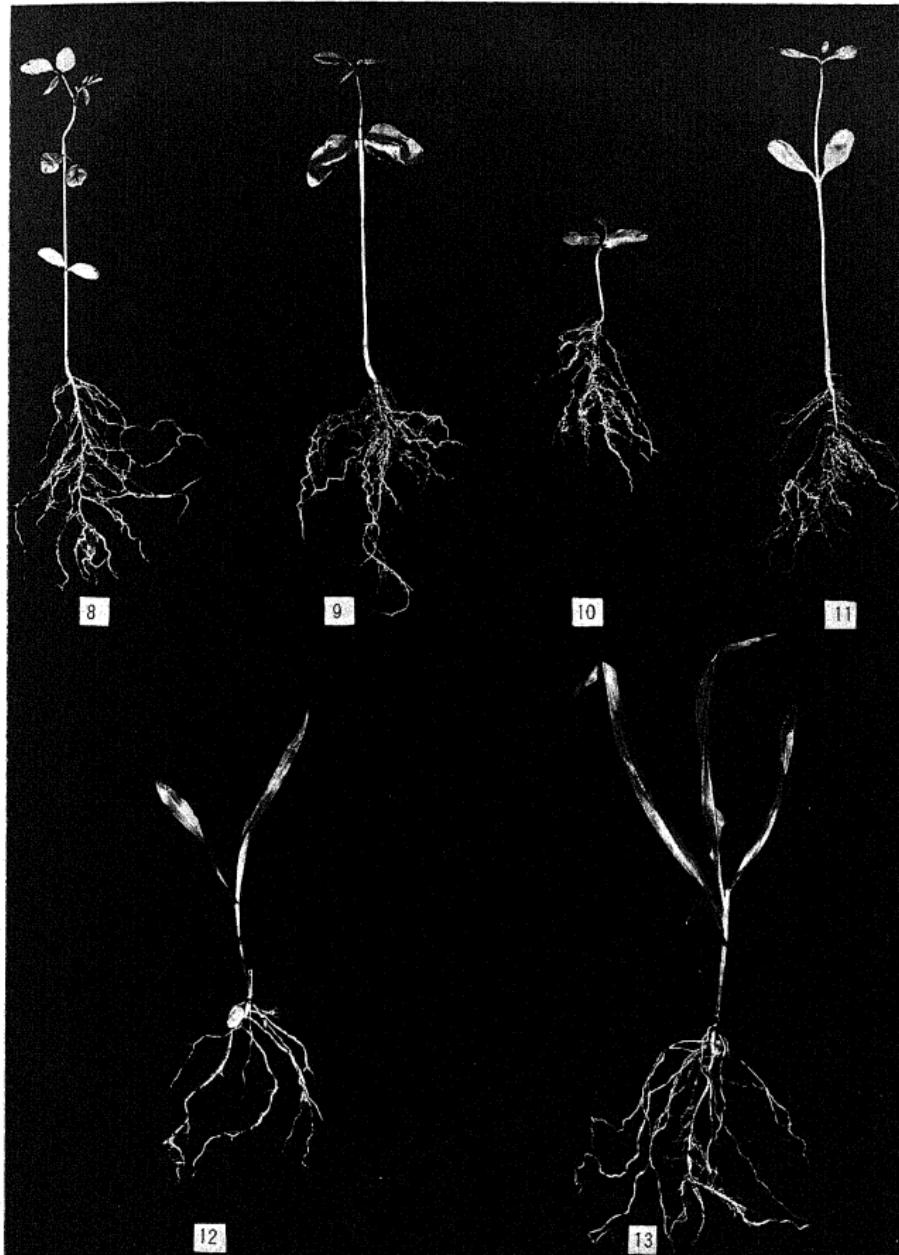
Sunflower seedlings grown in light:

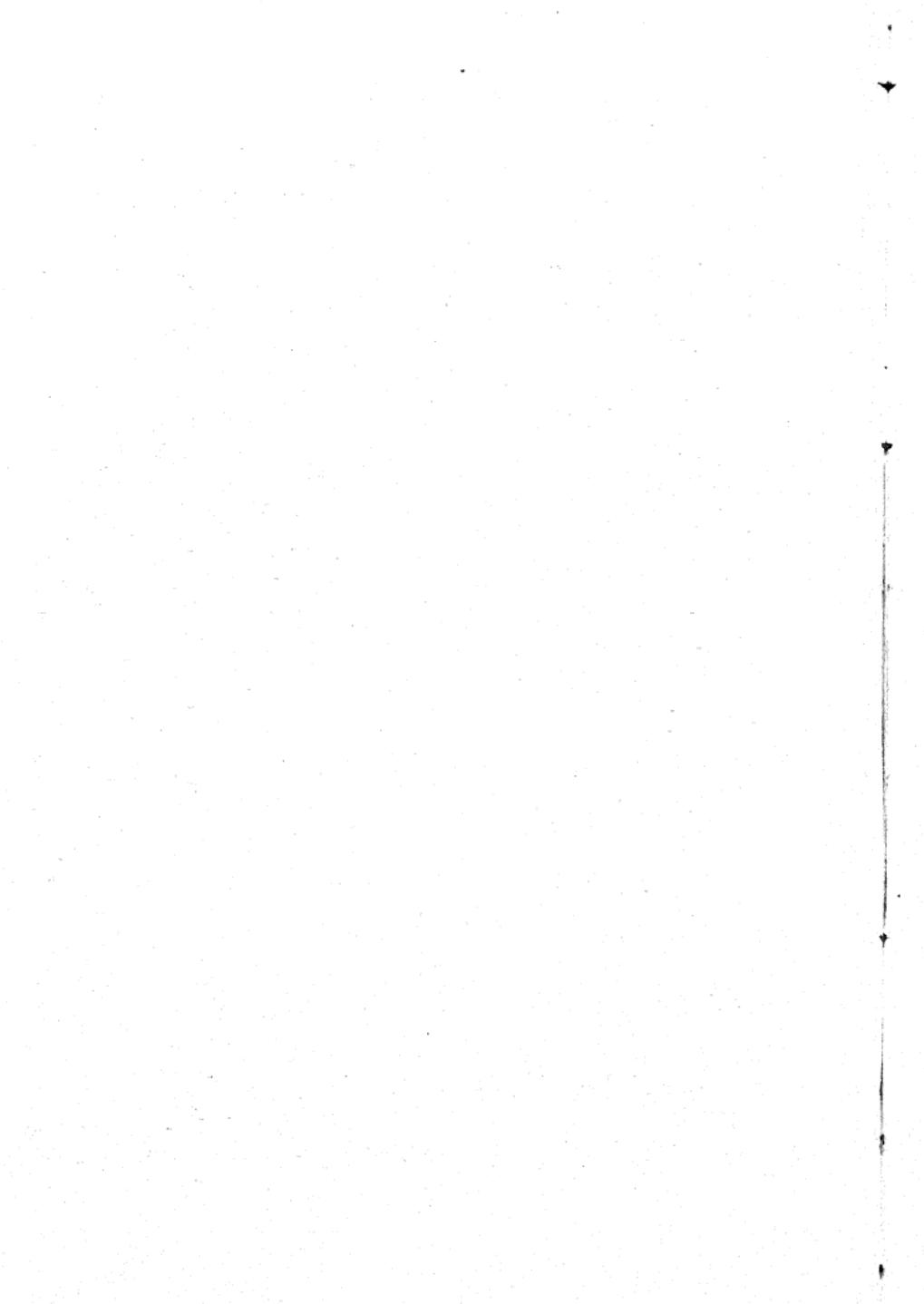
FIGS. 22, 24, 26, and 28.—In nutrient medium lacking nitrogen at stages of growth of one, two, three, and four weeks respectively.

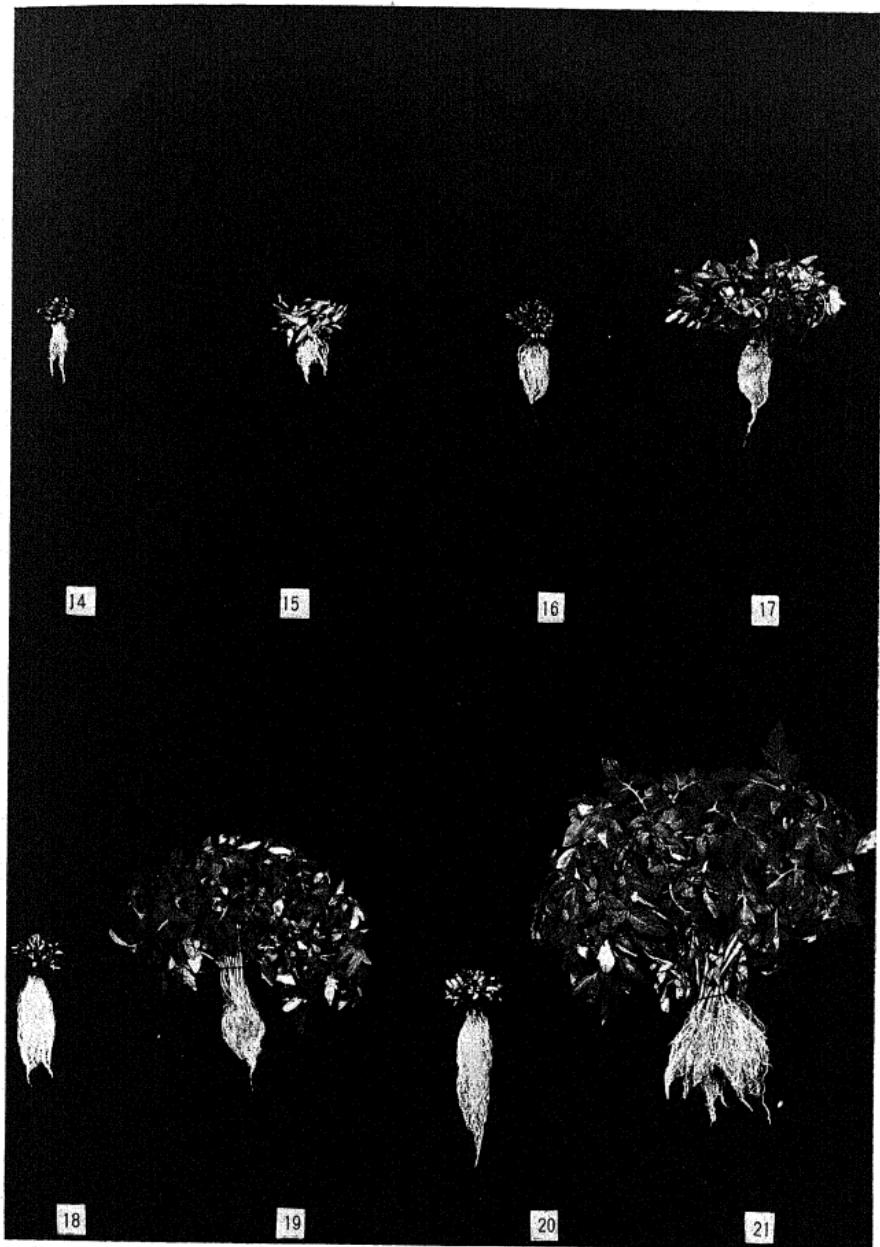
FIGS. 23, 25, 27, and 29.—In nutrient medium containing nitrates at stages of growth of one, two, three, and four weeks respectively.



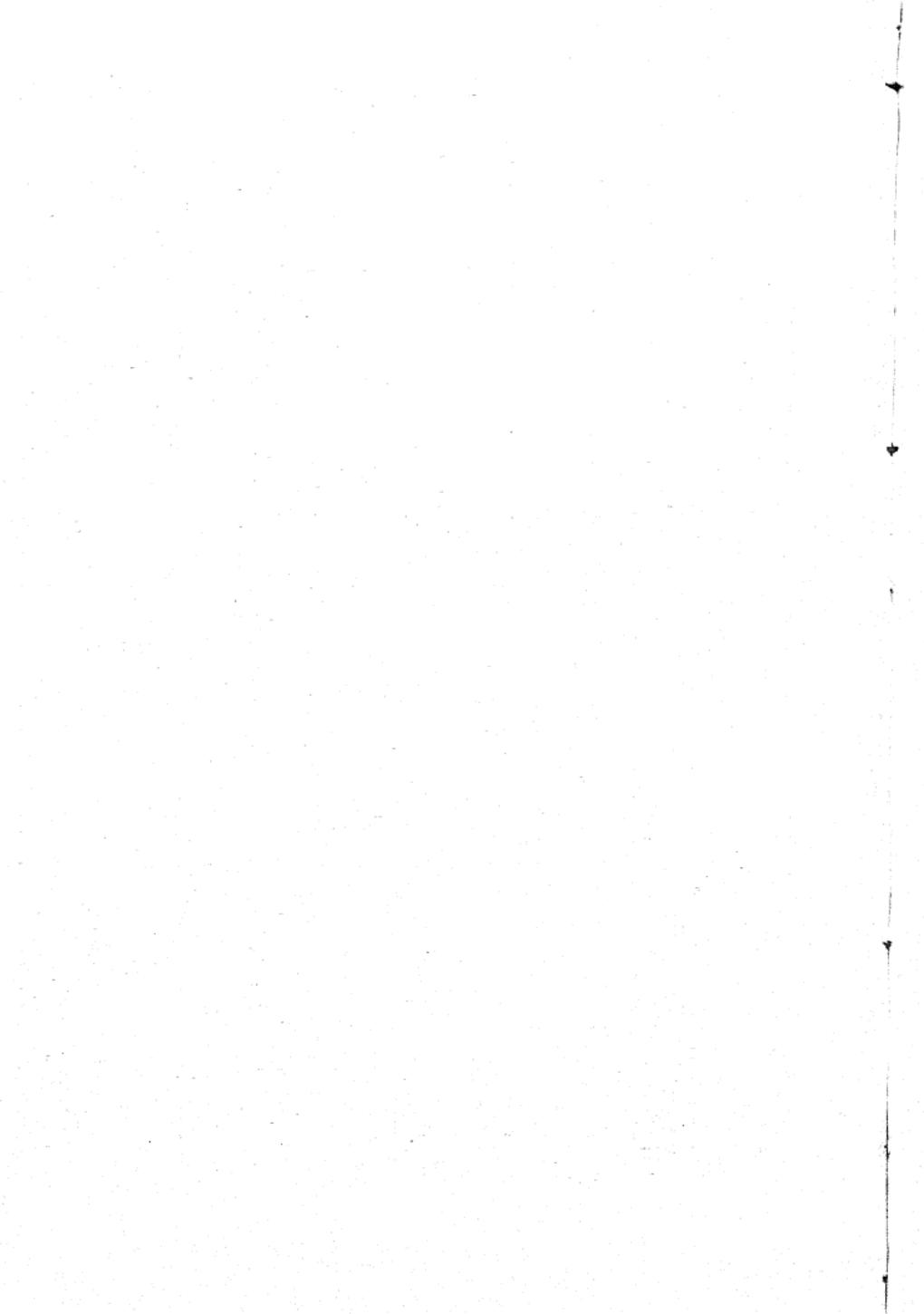








REID on SEEDLING GROWTH





REID on SEEDLING GROWTH

ORIGIN OF ADVENTITIOUS ROOTS IN COLEUS CUTTINGS¹

MARGERY C. CARLSON

(WITH PLATES V, VI)

Introduction

In connection with some microchemical work on the rooting of *Coleus* cuttings, it was necessary to know the origin of their adventitious roots. The extensive work of LEMAIRE (1) was concerned with the origin of naturally occurring endogenous adventitious roots in the hypocotyls, stolons, and rhizomes of many herbaceous dicotyledons. He grouped the plants studied into the following four classes, of which the first is most common: (1) all tissues of the root originating in the pericycle of the stem; (2) the central cylinder of the root coming from the pericycle, and the other regions from the endodermis and inner cortex of the stem; (3) all tissues of the root coming from the "subphloem meristem" (cambium); (4) the central cylinder of the root formed by the cambium, and the other tissues by the pericycle of the stem.

VAN TIEGHEM and DOUILLOT (5) added to their investigation of the origin of secondary roots a reinvestigation of the problem of the origin of natural adventitious roots in stems, chiefly hypocotyls and rhizomes. They came to the conclusion that endogenous roots arise entirely from the pericycle, except in older portions of stems where the pericycle has lost its "root-forming character." They define the pericycle as the layer, or layers, between the endodermis and the external phloem of a fibrovascular bundle, continuous across the medullary rays, but not distinguished on the inside from the medullary ray.

If the pericycle is simple, an arc of cells elongates radially and divides by tangential walls. The internal layer becomes the central cylinder of the new root. A tangential division of the external layer

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

separates the cortex (internal) from the epidermis (external). The cells of the epidermal layer surrounding the tip of the root divide again to form the root cap. If the pericycle is compound, it is usually its external layer which produces the epidermis, cortex, and central cylinder of the root. The other layers produce only the internal region of the central cylinder. When the root arises after the pericycle has lost its "root-forming" property, it may originate in the external primary phloem parenchyma, the internal primary phloem parenchyma, or the secondary phloem parenchyma.

Some recent work has dealt with the origin of adventitious roots in cuttings. SMITH (2), using cuttings of *Coleus blumei*, reports that "the first recognizable sign of the development of an adventitious root is the appearance in the cambium of a nest of highly meristematic cells." VAN DER LEK (4) described "root germs," present in young branches of *Ribes nigrum*, *Salix*, and *Populus*, as being "in connection with the cambium" as a "continuation of a medullary ray." SWINGLE (3) finds "root germs" initiated in the cambium ring of apple stems which produce burrknots.

Materials and methods

The work here reported was restricted to a study of the origin and development of adventitious roots arising between the fibro-vascular bundles in young stem cuttings of *Coleus blumei*.

The cuttings, 2-6 inches long, were made from the tips of branches of *Coleus* plants and placed with their cut ends in water or in sand. The cuts were usually made through the internodes. The basal portions of these cuttings were removed and preserved at intervals beginning with the third day. These pieces of stems were fixed in formalin-acetic-alcohol and imbedded in paraffin. Sections, both transverse and longitudinal, were cut 12-15 μ in thickness and stained with safranin and gentian violet. In all cases serial sections were studied, and the median section of the primordium or young root was used for the figures.

In this paper the terms "origin" or "initial" will be used to indicate the cell, or group of cells, which by division initiate an adventitious root; "root primordium" to mean the group of meristematic cells from the time of earliest divisions of the "origin" to the

time of differentiation into well marked regions characterizing a young root.

Observations

The structure of the stem of *C. blumei* has been described by SMITH (2). The arrangement and differentiation of the tissues in the stems used in this work are shown in figs. 1, 3, and 5. The outer cortex at the stages shown has not yet developed into collenchyma. The endodermis is a somewhat regular layer of cells, usually smaller than the typical cortical parenchyma cells, and is easily detected because of the presence of large, compound starch grains (fig. 4). The pericycle consists of a single layer of cells just inside the endodermis. In stems older than those used for this study the pericycle opposite the vascular bundles differentiates into sclerenchyma.

Four large fibrovascular bundles occupy the angles of the stem. One or two smaller bundles lie between each two contiguous corner bundles (fig. 7). Isolated groups of primary phloem cells occur between the bundles. Such groups are seen in figs. 1 and 3. The layer of pericycle cells lies just outside these groups of cells.

A continuous cylinder of cambium was well developed in all the stems studied. In some cases the cambial cells had just begun to divide to form secondary xylem and phloem (fig. 7), but more commonly three to five layers of secondary xylem and one or two layers of secondary phloem were already present (figs. 1, 3, and 5). The interfascicular cambium arises one or two layers inside of the pericycle. There are, therefore, one or two layers of parenchymatous cells between the pericycle and the interfascicular cambium.

SMITH (2) finds that "the first roots appear in four ranks, corresponding to the four vascular strands. This arrangement may be obscured later by roots which arise irregularly in between." As a rule, the findings reported in this paper are in agreement with SMITH's observations.

Table I shows the age and position of root primordia and adventitious roots in seven cuttings chosen at random. The roots are numbered in the order of their appearance. The oldest root, that is, the first to appear, is numbered 1; the next younger, 2, etc. The numbers are arranged in columns with respect to the position of the roots in the stem from the cut surface upward; the lowest number

in a column therefore represents the lowest root in the stem, and so on. The letters following the numbers indicate the position of the adventitious roots with respect to the fibrovascular bundles. "C" indicates that the root appears opposite a large corner bundle; "EC," at the edge of a corner bundle; "L," opposite a lateral bundle; "B," between two bundles.

TABLE I

ORDER OF APPEARANCE AND POSITION OF ROOTS AND ROOT
PRIMORDIA IN SEVEN COLEUS CUTTINGS
(EXPLANATION IN TEXT)

| CUTTING NUMBER | | | | | | |
|---|---------------------------------|------------------------------------|---------------------------------|----------------------------------|--|---|
| I | II | III | IV | V | VI | VII |
| 1-C 2-C 3-L 4-C 6-EC 5-C | 1-C 5-C 3-B 2-C 4-C | 3-EC 2-EC 1-C 4-EC 1-C | 1-C 2-C 3-C 1-C 4-B | 1-C 5-EC 3-C 4-C 4-C | 1-B 6-L 6-B 7-L 2-C 7-L 2-C 4-C 8-B 5-EC 3-C | 3-C 5-B 2-EC 3-C 1-C 3-C 6-B 7-B 6-B 5-B 4-EC 5-B 10-B 6-B 10-B 6-B 10-B 7-B 8-B 9-B 10-B |

In general, the first four or five roots arose opposite corner bundles, while later roots arose opposite lateral bundles or between them. An exception was found in four of the cuttings examined. In cutting VI the first root arose between the bundles; in cutting V, the second; in cutting III, the third; and in cutting IV, the fourth.

There was no regularity in the appearance of the roots with respect to the cut surface. Sometimes the first roots arose nearest the cut surface (table I, cuttings III and IV), and sometimes farthest from the cut surface (cuttings I, II, IV, V, VI, and VII). All roots appeared within a distance of 2-3 mm. from the base of the cuttings.

The first evidence of adventitious root formation is an accumulation of protoplasm and an increase in the size of the nucleus and of the nucleole in one cell or in several adjacent cells of the pericycle. The enlarged nucleus takes a central position. The cell or cells then divide.

Fig. 1 shows three neighboring pericyclic cells which have divided, thus initiating an adventitious root. The cell on the left divided tangentially, and then the inner daughter cell divided tangentially. The middle cell of the group divided by a tangential wall; its inner daughter cell divided by a tangential wall and then the outer granddaughter cell divided slightly obliquely. The first division of the cell on the right of the group was also tangential.

In fig. 2 a pericyclic cell has divided either tangentially or radially, and its daughter cells either radially or tangentially. The cell on its left has divided into two very unequal daughter cells by an oblique wall in the upper corner. Several adjoining pericyclic cells on the right have each divided tangentially.

From a study of serial sections of the primordium shown in fig. 3, it was evident that the first division of the initial pericyclic cell was radial. Each daughter cell then divided tangentially into two unequal cells, the inner one being larger than the outer. The inner left cell must have divided transversely and one of its daughter cells then radially, the other obliquely. Only the cell with the oblique wall shows in the figure. Divisions had proceeded further in the two outer granddaughter cells. The one on the right divided transversely and its upper daughter cell radially into two unequal cells.

In other cases studied the division of the cells of the root origin was radial, or occasionally oblique, but usually the division was tangential. The divisions of the daughter cells may be tangential, transverse, or radial. LEMAIER (1) and VAN TIEGHEM and DOULIOT (5) found that the initial cells of a primordium always divided tangentially, and that the external layer of daughter cells always divided again tangentially, thereby making three layers of cells which developed into the three regions of the young root. In the work herewith reported no such regularity in development was found.

Cell divisions continue without much enlargement of the daughter cells before their divisions, until the space occupied by the original

one (or several) active pericyclic cells is filled by a large number of small, more or less cubical, meristematic cells (figs. 5-7).

Pericyclic cells adjacent to the initial and parenchymatous cells toward the inside of the initials (fig. 5) begin to divide and become a part of the root primordium. The history of the divisions of these cells is similar to that of the initial cells. In fig. 11, for example, the cell on the extreme left of the primordium has divided either tangentially and its daughter cells radially, or radially and its daughter cells tangentially. The cell lying next to it toward the right has divided tangentially; its daughter cells have divided tangentially. The two inner granddaughter cells divided radially. The cell to the right of the median cell in the primordium shown in fig. 12 has probably divided first radially, then each of its daughter cells tangentially, and each of the granddaughter cells again tangentially, forming two regular radial rows of four cells each.

Further development of the primordium consists in continued cell divisions, enlargement of the cells, and the addition of other neighboring cells (figs. 8-12). It is impossible to trace the order of cell divisions in the older primordia. The cells which resulted from the division of the median initial cell begin first to enlarge (fig. 9). The cells from adjacent initial cells follow, and as enlargement of the cells proceeds the primordium bulges toward the outside (figs. 9-12). The cells of the endodermis divide radially to allow for the push from the developing primordium (figs. 9, 12).

Figs. 7 and 8 show the position of the root primordia in relation to the lateral fibrovascular bundles. The pericycle can be followed outside the primary phloem of the bundle. The interfascicular cambium is continuous with the cambium of the bundle. It is evident that the cambium has taken no part in the formation of the primordium. Several rows of undivided cells lie between the young primordium and the cambium.

The position of the young primordia in relation to the groups of primary phloem cells is best seen in fig. 3.

There is no evidence of differentiation into the epidermis, root cap, cortex, and central cylinder of a root in any of the stages of development previous to that shown in fig. 12. All of the cells of the primordium seem to be equally capable of division. Several nuclei in process of division can be seen in fig. 12.

Fig. 13 shows a root primordium which has become definitely hemispherical and has begun to differentiate into the tissues of a root. The single external layer of cells becomes the epidermis. Two or three layers of cells inside the epidermis become the cortex and the innermost region becomes the central cylinder. A slightly older root (fig. 14) shows the beginning of the root cap from the tangential division of the epidermal cells which surround the apex.

The further development of the root has been adequately described by former investigators. Elongation proceeds by the division of cells in all parts of the young root, and by elongation of the cells of the central cylinder. The endodermis incloses the developing root for a short time, then breaks or dissolves. The cavity opposite the apex of the root (figs. 13, 14) indicates that the cells of the cortex are being dissolved before the growing root. Finally the tissues of the central cylinder differentiate, connection is made with the vascular system of the stem, and the root emerges.

Summary

1. Adventitious roots arising between the fibrovascular bundles from the bases of young cuttings of *Coleus blumei* originate in one to several adjacent cells of the pericycle.
2. The first division of the initial cells of a root is usually tangential, but it may be radial, or even oblique.
3. During the early stages of development the daughter cells divide without first increasing in size, so that a young primordium consisting of many cells may occupy the same space as the pericyclic cells from which it originated.
4. The primordium enlarges and bulges into the cortex before differentiating into the tissues of a root.
5. Subsequent development is precisely as described by the early investigators of the subject of root development.

The writer wishes to express her appreciation to Dr. C. E. ALLEN, Department of Botany, University of Wisconsin, Madison, Wisconsin, for criticisms on the manuscript while in preparation.

NORTHWESTERN UNIVERSITY
EVANSTON, ILL.

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EXPLANATION OF PLATES V, VI

All figures are photomicrographs. The walls of the cells and the nuclei were traced with India ink, and the photographs then bleached. The plates were reduced one-fourth. Figs. 1-12 are transverse sections of stem cuttings; figs. 13 and 14 are longitudinal sections of stem cuttings. All sections of root primordia except fig. 11 are median.

PLATE V

FIG. 1.—Transverse section of stem, showing very young root primordium: *e*, epidermis; *c*, cortex; *en*, endodermis; *p*, pericycle; *pph*, primary phloem; *sph*, secondary phloem; *ca*, cambium; *sx*, secondary xylem; *pi*, pith.

FIG. 2.—Young root primordium.

FIG. 3.—Young root primordium, showing its relation to group of primary phloem cells.

FIG. 4.—Young root primordium; starch grains in endodermis.

FIG. 5.—Young root primordium, showing adjacent pericyclic cells and parenchymatous cells beginning to divide.

FIG. 6.—Root primordium, later stage than fig. 5.

FIG. 7.—Root primordium, showing its relation to lateral fibrovascular bundle.

PLATE VI

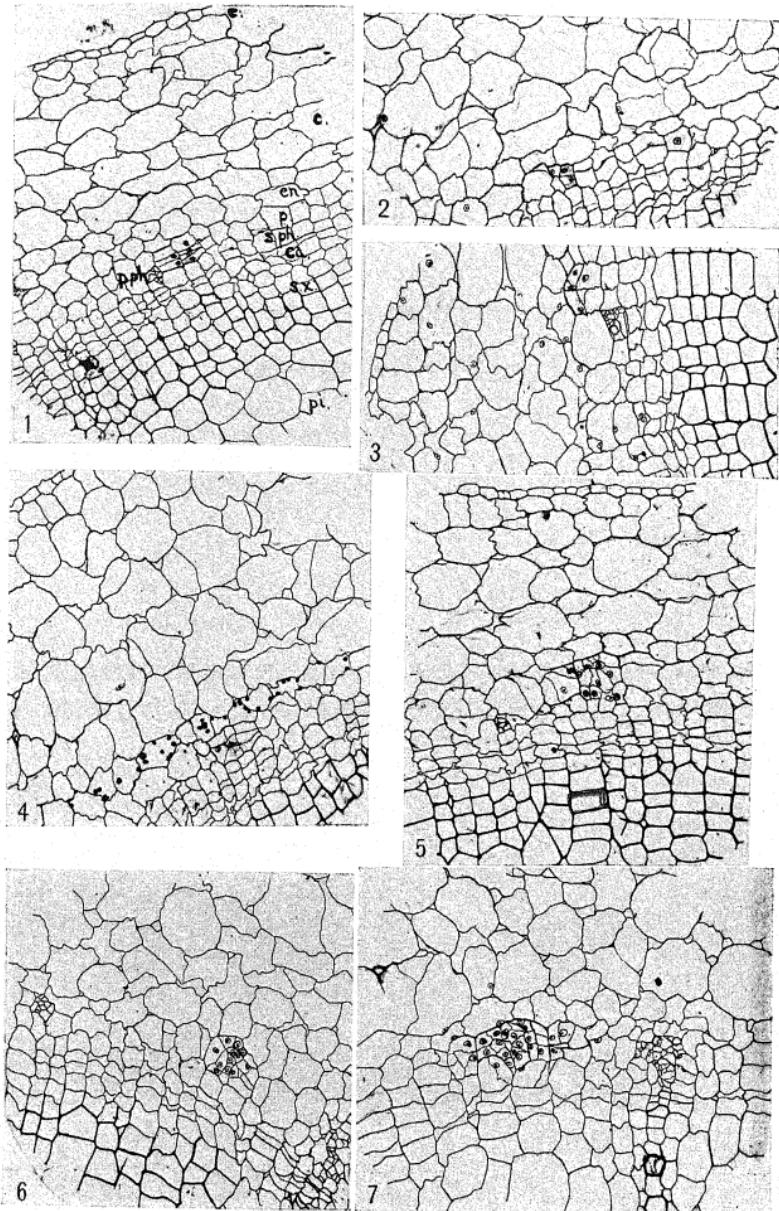
FIG. 8.—Root primordium, consisting of many cells, but occupying little more space than cells from which it originated.

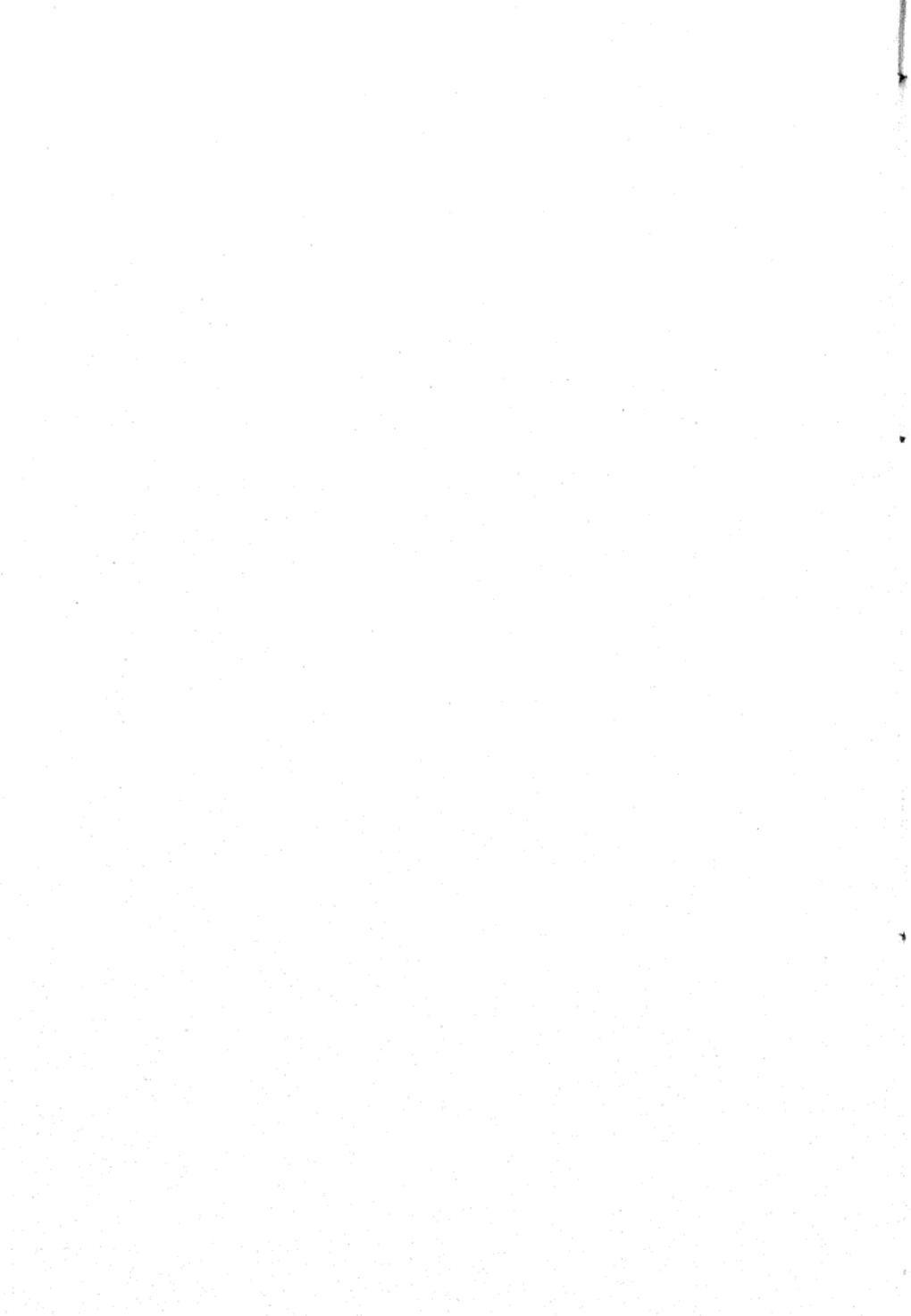
FIG. 9.—Root primordium, beginning to bulge into cortex; endodermal cells bordering primordium have divided; cambium has taken no part in production of primordium.

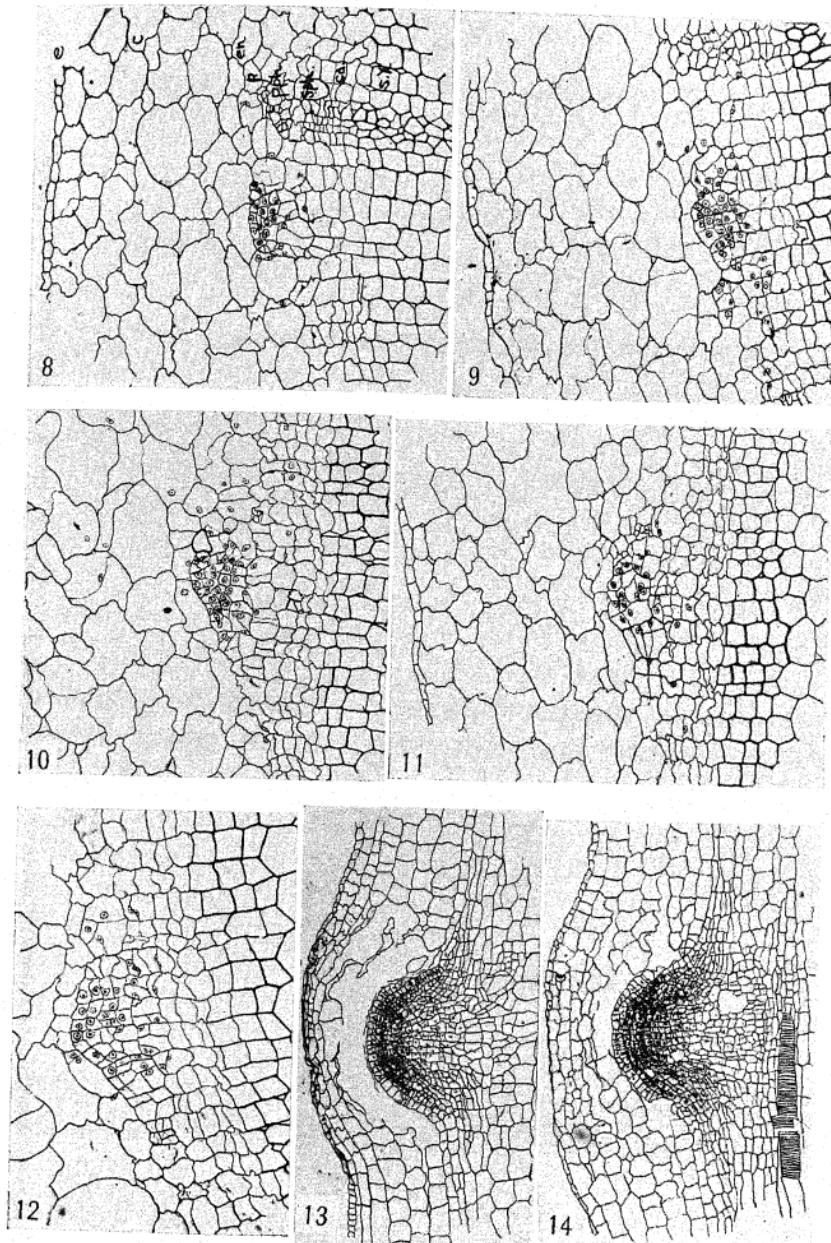
Figs. 10-12.—Older primordia.

FIG. 13.—Young root in which differentiation into epidermis, cortex, and central cylinder has begun; endodermis and part of cortex dissolved.

FIG. 14.—Young root, later stage than fig. 13; root cap beginning to form.







GERMINATION AND VITALITY OF BIRCH SEEDS¹

HILDA C. JOSEPH

(WITH FIVE FIGURES)

Introduction

In a recent paper, WEISS (13) has given his results with experiments on temperature and medium requirements for the germination of fresh and after-ripened seeds of *Betula populifolia*. He finds that the germination of *B. populifolia* is greatly improved when the seeds are stored in moist granulated peat at low temperature for about two months; that 10° C. is as effective as 5° C. or 0° C. for this purpose; that germination percentage is increased by a treatment with an organic mercury disinfectant previous to stratification; and that such after-ripening at low temperatures results in a marked downward shift in the minimum temperature required for germination.

In the work reported in this paper, the writer has extended studies of the same type to other species of *Betula*, and has also tried to determine whether seeds of the same species vary in their behavior when collected at different times of the season and when kept in different conditions of storage for a year or more.

Material and methods

The main studies have been with *Betula lenta* seeds, which were collected from trees in the forest of the Boyce Thompson Institute for Plant Research. September, October, and November collections were made from the same trees. Since the seeds of the October collection were superior to all others, they were used exclusively in the storage experiments.

The seeds of *Betula populifolia* used in these experiments were also collected from the Institute's forest, while *B. papyrifera* and *B. lutea* were commercial seeds, harvested and shipped by seed deal-

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

ers in October, 1926. All seeds were freed from wings by treatment in a Hobart mixer before being used.

In making the germination tests, Petri dishes with three layers of filter paper were employed, except when another substratum is indicated. Two hundred seeds were placed in each dish.

In the early determinations on the optimum conditions for after-ripening, that is, for the improvement of germination through temporary moist storage at cool temperatures, the seeds were mixed with moist acid granulated peat, neutralized granulated peat, or sand, and placed in large-mouthed bottles covered with cheesecloth.

TABLE I
CHANGE OF CONCENTRATION IN SOLUTIONS OF SULPHURIC ACID USED
TO REGULATE MOISTURE CONTENT OF *BETULA* SEEDS

| CONCENTRATION OF H_2SO_4 (PERCENTAGE) | SPECIFIC GRAVITY AT BEGINNING OF EXPERIMENTS | SPECIFIC GRAVITY OF FIRST SET OF SOLU- TIONS AT END OF FIRST 2 WEEKS | SPECIFIC GRAVITY OF SECOND SET OF SOLUTIONS AT END OF SECOND 2 WEEKS |
|---|--|---|---|
| Concentrated... | | | |
| 64.8 | 1.8300 | 1.8046 | 1.8181 |
| 55.9 | 1.5200 | 1.5050 | 1.5024 |
| 43.4 | 1.4300 | 1.4029 | 1.4185 |
| 30.4 | 1.3300 | 1.3042 | 1.3200 |
| 18.4 | 1.2234 | 1.2021 | 1.2092 |
| | 1.1210 | 1.0334 | 1.1890 |

In the later studies the seeds were after-ripened in lots of 200 on filter papers in Petri dishes. This latter method proved just as effective and more practical for these studies, since the after-ripened seeds could then be used in germination tests without previous transfer from the bottles to Petri dishes.

The moisture determinations were made by drying non-macerated seeds to constant weight at 103° C. When seeds were prepared for dry storage at various moisture contents, the samples were brought to water vapor equilibrium with a given concentration of sulphuric acid in a desiccator, which was provided with a stirrer for both the air and the sulphuric acid solution. The solutions were changed every two weeks. The changes in specific gravity of the sulphuric acid solutions that occurred during the first two periods are given in table I.

After eight weeks of storage over H_2SO_4 solutions, the seeds had

reached water equilibrium with the solutions. At this time one series of samples was taken out to determine the moisture content of the different lots of seeds; a second series was taken out for the purpose of germination tests; the third and largest amount of material was placed in small bottles, sealed air-tight, and stored under the following conditions: at room temperature, in an ice chest at about 8° C., and in a room varying in temperature from -15° to -8° C. A different method of storage was employed with seeds that had been exposed to humidified atmospheres over sulphuric acid solutions of concentrations lower than 55.9 per cent. Here the seeds were sus-

TABLE II
WEIGHT AND MOISTURE CONTENT OF VARIOUS COLLECTIONS OF *BETULA*
SEEDS AS DETERMINED IMMEDIATELY AFTER HARVEST OR AFTER
ARRIVAL OF SHIPMENT

| COLLECTION | WEIGHT OF 1000 SEEDS AIR-DRY (GM.) | WEIGHT OF 1000 SEEDS DRIED TO CONSTANT WEIGHT (GM.) | MOISTURE CONTENT IN PERCENTAGE DRY WEIGHT |
|--|--|--|--|
| <i>Betula lenta</i> , collection A and B, September, 1926..... | 0.7300 | 0.6730 | 7.54 |
| <i>Betula lenta</i> , collection, October, 1926..... | 0.5471 | 0.5084 | 7.09 |
| <i>Betula lenta</i> , collection, November, 1926..... | 0.4863 | 0.4570 | 6.04 |
| <i>Betula populifolia</i> , collection, October, 1926..... | 0.0920 | 0.0853 | 7.36 |
| <i>Betula papyrifera</i> , commercial collection, 1926..... | 0.2740 | 0.2502 | 8.70 |

pended in a wire basket from the cover of a tightly closed museum jar over the different solutions, and stored in this way at room temperature and in an ice chest. In the low temperature room all seeds were stored in tightly sealed bottles. The total number of seeds of the various species and collections used in these studies approximates one million.

Experimental results

I. EXPERIMENTS WITH NEW SEEDS

It is common practice among gardeners and nurserymen to keep freshly harvested seeds on drying racks for a short time before they are planted. This is done mainly to avoid molding and fermentation in seeds surrounded by a fleshy pulp; but there are also cases in which a period of drying is directly beneficial to the germination

quality of the seed material. Wheat and other grains, especially if they have ripened during a rainy season, are improved by a period of dry storage.

It seemed to be of interest to determine whether such an improvement of germination quality could also be obtained in *Betula* seeds, and whether it could be obtained in seeds picked at the beginning of the harvesting season as well as in those picked at the end. Table II shows that the percentage of hygroscopic moisture of the seeds decreases with the advancing season, and that the weight for 1000 seeds is lower in the later collections.

TABLE III
GERMINATION OF FRESHLY HARVESTED *BETULA LENTA* SEEDS
IMMEDIATELY AFTER HARVEST

| DESCRIPTION OF MATERIAL | NO. USED IN EACH TEST | PERCENTAGE GERMINATION AT | | | |
|--|-----------------------------|---------------------------|--------|--------|--------|
| | | 15° C. | 20° C. | 25° C. | 32° C. |
| Collection A, September, 1926, from green catkins..... | 500 | 0 | 5 | 3 | 7 |
| Collection B, September, 1926, from brown catkins..... | 500 | 0 | 2.5 | 5 | 3.5 |
| Collection, October, 1926..... | 200 | 0 | 0 | 0 | 17 |
| Collection, November, 1926..... | 200 | 0 | 0 | 1 | 2 |

The September collection was taken from closed catkins which were dried in the laboratory to induce shedding of the seeds. The October and November collections were taken from catkins that had already opened on the trees. The November collection had also been exposed to some freezing on the trees. The data in tables III and IV show that the germination quality is improved considerably in all the different collections through one month of dry storage at laboratory temperature. This improvement, therefore, must be due to changes in the seed different from those which are produced by nature through variations in humidity, temperature, and ventilation during the period in which the seed hangs on the tree.

Since acids have been found to have a favorable influence upon the germination of some seeds, it was thought possible that an acid

substratum for freshly harvested seeds might substitute for the beneficial effects of dry storage. Table V shows the negative results

TABLE IV
GERMINATION OF *BETULA* SEEDS AFTER ONE MONTH OF
DRY STORAGE IN LABORATORY

| DESCRIPTION OF MATERIAL | NO. USED IN EACH TEST | PERCENTAGE GERMINATION AT | | | | |
|---|--------------------------|---------------------------|--------|--------|--------|---------------------------------|
| | | 15° C. | 20° C. | 25° C. | 32° C. | Alternation of 15°-32° C. |
| B. lenta, collection A, September, 1926 | 200 | 0 | 0 | 0 | 17 | 48 |
| B. lenta, collection October, 1926 | 400 | 0 | 0 | 0 | 41 | 46 |
| B. lenta, collection November, 1926 | 200 | 0 | 0 | 0 | 11 | 39 |
| B. papyrifera, commercial collection 1926 | 600 | 0 | 9 | 67 | 80.5 | 81 |
| B. populifolia, collection October, 1926 | 200 | 0 | 17 | 51 | 60 | 58 |
| B. lutea, commercial collection, 1926 | 400 | 0 | 2 | 3 | 8 | 15 |

TABLE V
INDIFFERENCE OF FRESH *BETULA LENTA* SEEDS TO ACIDITY OF SUBSTRATUM

| DESCRIPTION OF MATERIAL | NO. USED IN EACH TEST | SUBSTRATUM | PERCENTAGE GERMINATION AT | | | |
|--|--------------------------|---|---------------------------|--------|--------|--------|
| | | | 15° C. | 20° C. | 25° C. | 32° C. |
| Collection A, September, 1926 from green catkins | 500 | Filter paper | 0 | 0 | 0 | 4.6 |
| | 500 | Peat pH 4.6 | 0 | 5.4 | 4 | 6 |
| | 500 | Peat, neutral with excess CaCO_3 | 0 | 1 | 0 | 5.8 |
| Collection B, September, 1926 from brown catkins | 500 | Filter paper | 0 | 1 | 0 | 5 |
| | 500 | Peat pH 4.6 | 0 | 3 | 5 | 3.5 |
| | 500 | Peat, neutral with excess CaCO_3 | 0 | 0 | 4 | 5 |
| Collection October, 1926 | 200 | Filter paper | 0 | 0 | 1 | 17 |
| | 200 | Peat, neutral with excess CaCO_3 | 0 | 0 | 0 | 2 |

obtained with tests arranged for this purpose. Except for a probable slight improving of germination on acid peat at 20° C. of the earliest collection of *B. lenta*, there is no noticeable effect that could be interpreted as favorable influence of the organic acids of peat upon the germination quality of fresh seeds.

Similar tests were conducted to determine whether a favorable

effect produced by light, or by increased or reduced oxygen pressure, could be observed, but the seeds were not sensitive to considerable variations in intensity of these factors. A short time later the same experiments were repeated with laboratory-stored seeds with the same negative results.

A decrease of germination percentage occurred in laboratory-stored seeds of *B. lenta* after sterilization with 0.25 per cent uspulun, as shown in table VI.

Although laboratory-stored seeds germinate well at high temperatures, as 32° C. and at alternation of 15° - 32° C., they do not germinate

TABLE VI

EFFECT OF STERILIZATION WITH USPULUN (0.25 PER CENT FOR ONE-HALF HOUR) UPON GERMINATION OF LABORATORY-STORED SEEDS OF *BETULA LENTA*; 200 SEEDS IN EACH TEST

| DESCRIPTION OF MATERIAL | GERMINATION AT FAVORABLE TEMPERATURE | | | |
|---|--------------------------------------|--------------|---|--------------|
| | 32° C. | | Alternation of 15° - 32° C. | |
| Collection October, 1926, sterilized... | 68 | Average 66.1 | 53 | Average 51.5 |
| | 63 | | 50 | |
| | 68 | | | |
| Collection October, 1926, not sterilized..... | 73 | Average 76 | 58 | Average 63.5 |
| | 81 | | 59 | |
| | 74 | | | |

nate to a considerable percentage at constant temperatures below 32° C. By a suitable period of moist storage at cool temperatures this behavior can be changed. The seeds are "after-ripened" through this treatment, and afterward germinate better, not only at higher temperatures, but also at temperatures as low as 15° C. If kept in cool storage continuously they finally even germinate at 0° C. All species studied react in a similar way, although *B. papyrifera* is the least dormant of the species studied; and the improvement in total germination percentage, as well as in enlarging of the temperature range favorable to germination, is therefore less pronounced. The seeds of *B. lutea* seemed to be of low vitality from the start, so that differences in behavior under various conditions were less noticeable for this species.

Tables VII-X show the effect that various moist storage condi-

tions have upon the germination of *Betula* seeds at temperatures ranging from 15° to 32° C. Of the four different storage temperatures employed, 0° and 5° C. proved to be very much superior to 10° C., and to a constant frozen condition. This is shown by the high percentage of germination obtained at low germination temperatures

TABLE VII

GERMINATION OF *BETULA LENTA* SEEDS, COLLECTION OCTOBER, 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

| TEMPERATURE DURING PERIOD OF MOIST STORAGE | WEEKS IN MOIST STORAGE | PERCENTAGE GERMINATION AT | | | | Alteration of 15° – 32° C. |
|---|------------------------|---------------------------|-----------------|-----------------|-----------------|--|
| | | 15° C. | 20° C. | 25° C. | 32° C. | |
| Control (no moist storage)..... | | 10 | 0 | 2 | 33 | 67 |
| 0° C..... | 4 | 80 | 80 | 77 | 72 | 75 |
| | 6 | 85 | 72 | 82 | 75 | 81 |
| | 8 | 77 | 79 | 81 | 82 | 77 |
| | 10 | 82 | 79 | 77 | 76 | 60 |
| 5° C..... | 4 | 83 | 75 | 77 | 72 | 77 |
| | 6 | 70 | 81 | 57 | 72 | 77 |
| | 8 | 72 | 77 | 74 | 54 | 56 |
| | 10 | 85 | 76 | 87 | 82 | 82 |
| 10° C..... | 4 | 7 | 22 | 24 | 58 | 78 |
| | 6 | 2 | 4 | 25 | 30 | 74 |
| | 8 | 10 | 10 | 16 | 54 | 72 |
| | 10 | 2 | 4 | 26 | 60 | 71 |
| In frozen condition at -15 to -8° C. (soaked in water for a few hours at room temperature previous to storage)..... | 4 | 0 | 0 | 0 | 15 | 37 |
| | 6 | 0 | 0 | 1 | 28 | 69 |
| | 8 | 0 | 0 | 9 | 22 | 72 |
| | 10 | 0 | 2 | 31 | 28 | 55 |

after a period of moist storage at these temperatures. Seeds stored at 10° C. showed only a slight improvement of germination when transferred to the different germination chambers. For this reason 10° C. cannot be termed a good temperature for after-ripening of the species studied here. This observation differs from that of WEISS (13), who found that for the after-ripening of *B. populifolia* a moist storage period at 10° is as effective as one at 5° or at 0° C. Seeds stored below the freezing point failed completely to after-ripen.

When the samples were put into the cold chamber immediately after they had been placed on moist filter paper, so that no absorption of water could take place before the seeds were frozen, their quality was not altered at all when they were transferred into the germination chambers. When the seeds were allowed to absorb some moisture

TABLE VIII

GERMINATION OF *BETULA LENTA* SEEDS, COLLECTION NOVEMBER, 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

| TEMPERATURE DURING PERIOD OF MOIST STORAGE | WEEKS IN MOIST STORAGE | PERCENTAGE GERMINATION AT | | | | |
|--|------------------------|---------------------------|--------|--------|-----------------|----------------------------|
| | | 15° C. | 20° C. | 25° C. | 32° C. | Alter-nation of 15°-32° C. |
| Control (no moist storage)..... | | 1 | 0 | 4 | 4 | 12 |
| 0° C. | 4 | 45 | 22 | 13 | 6 | 17 |
| | 6 | 36 | 41 | 28 | 10 | 15 |
| | 8 | | | | Experiment lost | |
| | 10 | | | | Experiment lost | |
| 5° C. | 4 | 48 | 52 | 42 | 32 | 23 |
| | 6 | 42 | 45 | 36 | 29 | 37 |
| | 8 | 49 | 50 | 48 | 37 | 35 |
| | 10 | 80 | 50 | 48 | 32 | 38 |
| 10° C. | 4 | 37 | 22 | 44 | 18 | 45 |
| | 6 | 22 | 32 | 41 | 28 | 52 |
| | 8 | 46 | 30 | 41 | 28 | 38 |
| | 10 | 20 | 34 | 34 | 33 | 45 |
| Infrozen condition at -15° to -8° C. (soaked in water for a few hours at room temperature previous to storage)..... | 4 | 5 | 8 | 6 | 3 | 38 |
| | 6 | 7 | 3 | 6 | 3 | 19 |
| | 8 | 5 | 1 | 4 | 4 | 25 |
| | 10 | 8 | 8 | 4 | 1 | 55 |

ture, as in those samples recorded in tables VII-X, some injury could be noted in several samples. The amount of injury increased with the length of time the seeds were soaked at room temperature previous to freezing storage. An alternation of freezing and thawing killed samples of all three species. These results are not in agreement with those obtained by KINZEL (9) for various other seeds. He noted a beneficial effect of freezing and of an alternation between freezing and thawing.

The length of the cool moist storage period does not seem to be

of great importance. Four weeks proved to be long enough to after-ripen seeds at a favorable storage temperature, and ten weeks did not seem to be too long for a favorable result. In all later experiments six weeks of storage at 0°C . was selected as a suitable period for after-ripening.

TABLE IX

GERMINATION OF *BETULA PAPYRIFERA* SEEDS, COMMERCIAL COLLECTION 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

| TEMPERATURE DURING PERIOD OF MOIST STORAGE | WEEKS IN MOIST STORAGE | PERCENTAGE GERMINATION AT | | | | |
|---|------------------------|---------------------------------------|-----------------------|-----------------------|-----------------------|---|
| | | $15^{\circ}\text{C}.$ | $20^{\circ}\text{C}.$ | $25^{\circ}\text{C}.$ | $32^{\circ}\text{C}.$ | Alternation of 15° — $32^{\circ}\text{C}.$ |
| Control (no moist storage)..... | | 0 | 9 | 12 | 15.7 | 16.7 |
| $0^{\circ}\text{C}.$ | 4 | 20 | 15 | 21 | 13 | 19 |
| | 6 | 22 | 25 | 19 | 41 | |
| | 8 | 19.5 | 20 | 23 | 15 | 22 |
| | 10 | 29 | 20 | 23 | 21 | 19 |
| $5^{\circ}\text{C}.$ | 4 | 38 | 36 | 33 | 26 | 36 |
| | 6 | 36 | 35 | 35 | 30 | 34 |
| | 8 | 49 | 44 | 41 | 44 | 45 |
| | 10 | 53 | 50 | 49 | 39 | 50 |
| $10^{\circ}\text{C}.$ | 4 | 13 | 21 | 26 | 40 | 41 |
| | 6 | 19 | 25 | 20 | 33 | 52 |
| | 8 | 4 | 8 | 1 | 28 | 45 |
| | 10 | Not started, material partly infected | | | | |
| Infrozen condition at -15° to $-8^{\circ}\text{C}.$ (soaked in water for a few hours at room temperature previous to storage)..... | 4 | 0 | 8 | 4 | 7 | 7 |
| | 6 | 0 | 1 | 6 | 7 | 7 |
| | 8 | 0 | 7 | 4 | 8 | 10 |
| | 10 | 0 | 6 | 3 | 7 | 8 |

The superiority of the October collection of *B. lenta* seeds over the other collections of the same species, which had been noted in previous tests, became again noticeable when samples of the different collections were after-ripened and then germinated, as is shown in table XI. The conditions under which the October collection was harvested differed from those of the other collections in the following manner: the catkins containing the seeds were dry and partly opened, and a small quantity of the seeds had been shed, while the seeds of the September collection were taken from fresh catkins

which were still closed, and those of the November collection from dried catkins, from which the greater part of the seeds had been shed. The seeds of the November collection had also been exposed to several nights of severe frost, while those of the October collection were harvested before frost had set in. According to this, the optimum

TABLE X

GERMINATION OF *BETULA LUTEA* SEEDS, COMMERCIAL COLLECTION 1926, AT DIFFERENT TEMPERATURES AFTER PREVIOUS PERIODS OF MOIST STORAGE AT VARIOUS LOW TEMPERATURES FOR DIFFERENT LENGTHS OF TIME; 400 SEEDS IN EACH TEST

| TEMPERATURE DURING PERIOD OF MOIST STORAGE | WEEKS IN MOIST STORAGE | PERCENTAGE GERMINATION AT | | | | |
|---|------------------------|---------------------------|--------|--------|--------|----------------------------|
| | | 15° C. | 20° C. | 25° C. | 32° C. | Alter-nation of 15°-32° C. |
| Control (no moist storage)..... | 2 | 0 | 5 | 5 | 5 | 12 |
| 0° C..... | 4 | 26 | 27 | 20 | 17 | 13 |
| | 6 | 22 | 21 | 17 | 14 | 14 |
| | 8 | 20 | 22 | 18 | 11 | 17 |
| | 10 | 14 | 19 | 2 | 7 | 0 |
| 5° C..... | 4 | 5 | 10 | 11 | 12 | 17 |
| | 6 | 12 | 7 | 9 | 14 | 12 |
| | 8 | 11 | 11 | 10 | 12 | 10 |
| | 10 | 10 | 11 | 7 | 9 | 14 |
| 10° C..... | 4 | 5 | 5 | 7 | 8 | 16 |
| | 6 | 4 | 5 | 7 | 8 | 17 |
| | 8 | 4.5 | 4.5 | 6 | 9 | 15 |
| | 10 | 4.5 | 8 | 9 | 11 | 12 |
| In frozen condition at -15° to -8° C. (soaked in water for a few hours at room temperature previous to storage)..... | 4 | 0 | 1 | 5 | 5 | 3 |
| | 6 | 2 | 1 | 4 | 6 | 7 |
| | 8 | 1 | 1 | 3 | 4 | 7 |
| | 10 | 1 | 7 | 1 | 4 | 4 |

time for harvesting *B. lenta* seeds seems to be after the catkins have dried on the tree, but before they have opened far enough for a considerable part of the seeds to be shed.

All these experiments were conducted in the fall of 1926 and the spring of 1927, that is, during the first six months after harvest.

2. EXPERIMENTS WITH STORED SEEDS

In the fall of 1927 a new series of experiments was started for the purpose of determining the keeping quality of seeds from the same collections under various conditions. For these studies seeds

had been stored in open glass bottles at room temperature. They had also been kept, as previously described, with various amounts of hygroscopic moisture at room temperature, and in an ice chest with an average temperature of 8° C.

TABLE XI

GERMINATION OF DIFFERENT COLLECTIONS OF *BETULA LENTA* SEEDS AFTER-RIPENED FOR SIX WEEKS AT 0° C.; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

| COLLECTION | PERCENTAGE GERMINATION AT | | | | |
|------------------------------------|---------------------------|--------|--------|--------|---------------------------|
| | 15° C. | 20° C. | 25° C. | 32° C. | Alternation of 15°-32° C. |
| Collection A, September, 1926..... | 54 | 62 | 57 | 50 | 67 |
| Collection October, 1926..... | 85 | 72 | 82 | 75 | 81 |
| Collection November, 1926..... | 58 | 44 | 45 | 24 | 30 |

TABLE XII

GERMINATION OF *BETULA* SEEDS, STORED DRY AT LABORATORY TEMPERATURE FOR ONE AND ONE-HALF YEARS; SEEDS NOT AFTER-RIPENED; GERMINATION TESTS MADE IN SPRING OF 1928; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

| COLLECTION | PERCENTAGE GERMINATION AT | | | | |
|--|---------------------------|--------|--------|--------|---------------------------|
| | 15° C. | 20° C. | 25° C. | 32° C. | Alternation of 15°-32° C. |
| B. lenta, collection A, September, 1926, from green catkins..... | 0 | 0 | 0 | 15 | 50 |
| B. lenta, collection B, September, 1926, from brown catkins..... | 0 | 0 | 0 | 7 | 25 |
| B. lenta, collection October, 1926..... | 0 | 0 | 4 | 46 | 76 |
| B. lenta, collection November, 1926..... | 0 | 0 | 0 | 3 | 16 |
| B. papyrifera, commercial collection 1926..... | 0 | 11 | 15 | 16 | 19 |
| B. lutea, commercial collection 1926..... | 0 | 3 | 1 | 6 | 6 |

The results of the first sets of experiments are given in tables XII and XIII, showing the germination quality of the seeds which had been stored air-dry at room temperature for one and one-half years. They had not been protected against changes in temperature and atmospheric humidity during the entire storage period. Stored seeds, just like newly harvested laboratory-dry seeds, germinated well at 32° C., or an alternation of 15°-32° C., but showed an im-

provement in germination percentage and a great fall in the minimum germination temperature after a period of after-ripening. In figs. 1-3 the percentage of germination of new and stored seeds of various species and collections is compared. It is interesting to note that of the three collections of *B. lenta* made in 1926, the first and second kept their vitality perfectly, while the last collection lost it somewhat. This collection had the poorest quality from the beginning. The reason for this low vitality and poor keeping quality may be due to the period of rainy weather and several nights of frost to

TABLE XIII

GERMINATION OF *BETULA* SEEDS, STORED DRY AT LABORATORY TEMPERATURE FOR ONE AND ONE-HALF YEARS, THEN AFTER-RIPENED FOR SIX WEEKS AT 0° C.; GERMINATION TESTS MADE IN SPRING OF 1928; EACH PERCENTAGE IS AVERAGE OF TWO TESTS OF 200 SEEDS EACH

| COLLECTION | PERCENTAGE GERMINATION AT | | | | |
|--|---------------------------|--------|--------|--------|-----------------------------------|
| | 15° C. | 20° C. | 25° C. | 32° C. | Alter- nation of 15°-32° C. |
| <i>B. lenta</i> , collection A, September, 1926, from green catkins..... | 52 | 52 | 54 | 41 | 54 |
| <i>B. lenta</i> , collection B, September, 1926, from brown catkins..... | 20* | 18 | 19 | 15 | 17 |
| <i>B. lenta</i> , collection October, 1926..... | 81 | 83 | 86 | 78 | 77 |
| <i>B. lenta</i> , collection November, 1926..... | 44 | 34 | 33 | 16 | 25 |
| <i>B. papyrifera</i> , commercial collection 1926..... | 23 | 19 | 17 | 10 | 15 |
| <i>B. lutea</i> , commercial collection 1926..... | 3 | 5 | 4 | 3 | 4 |

* Some fungal infection in this collection.

which these seeds had been exposed on the tree; or to the fact that the heavier and better developed seeds had dropped out of the catkins before this last collection was made.

Of all the species studied, *Betula papyrifera* lost its vitality quickest. In one year of storage the germination dropped from 81 to 19 per cent at the optimum germination temperature. This is interesting in view of the fact that *B. papyrifera* has at the same time the least dormant seeds of the four species. The quality of *B. populifolia* seeds has improved in germination rather than decreased, while that of *B. lutea* is slightly lower.

It has been stated that all freshly harvested seeds improved equally during the first month of dry storage in the laboratory, re-

gardless of the amount of hygroscopic moisture present in the seed at the time of harvest. When the storage period was lengthened,

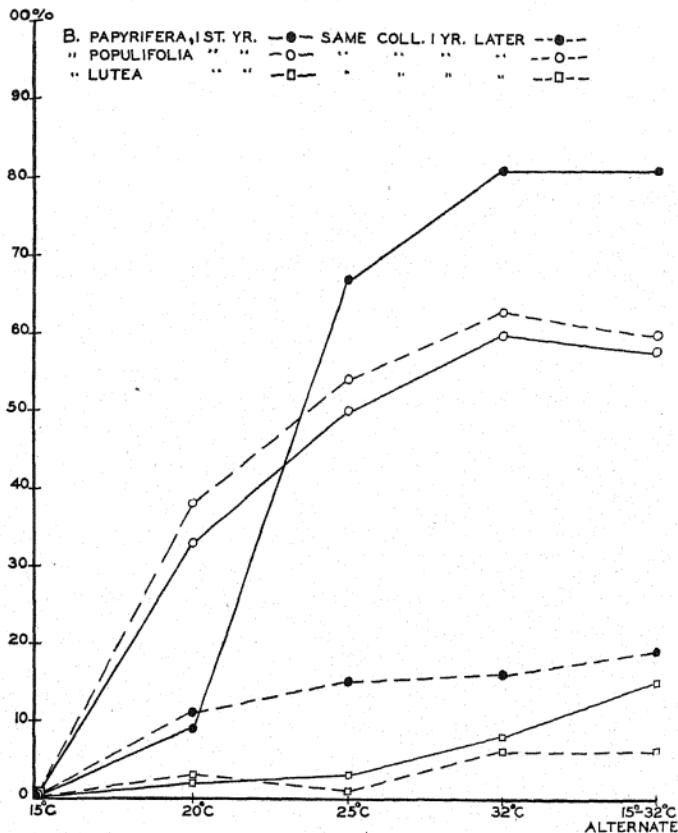


FIG. 1.—Germination of unafter-ripened *Betula* seeds at various temperatures shortly after harvest and after one year of storage at room temperature.

however, the amount of hygroscopic moisture contained in the various samples had a pronounced influence upon the changes going on in the seed during the storage period. Table XIV gives the water content expressed in percentage of dry weight of three species of

Betula as it was obtained by storage over CaO and different solutions of H_2SO_4 .

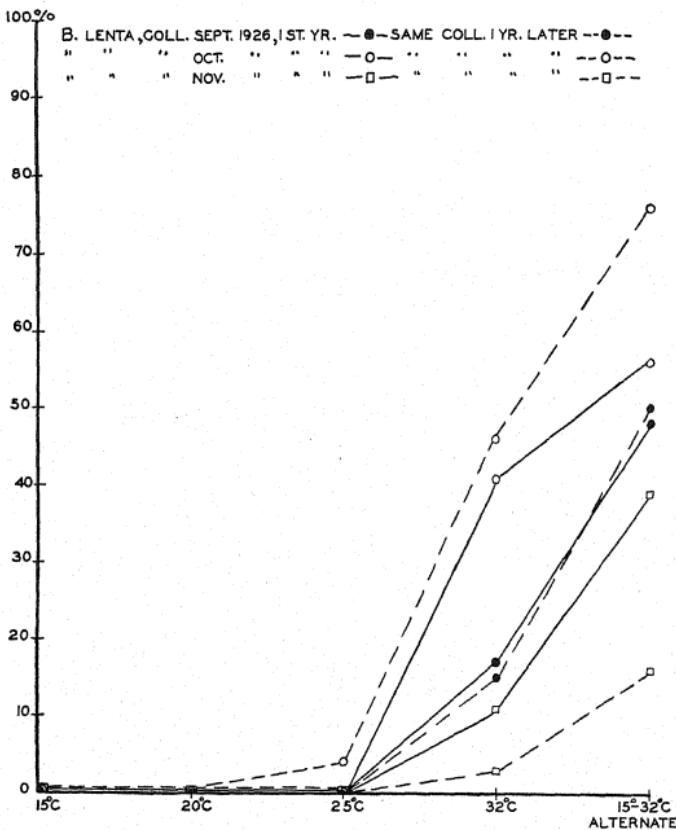


FIG. 2.—Germination of different collections of unafter-ripened *Betula lenta* seeds at various temperatures shortly after harvest and after one year of storage at room temperature.

The keeping quality of each sample from this series was determined for two different storage temperatures, room temperature and ice box temperature, and the results obtained are tabulated in tables

XV, XVI, and XVII. There was also one series of samples stored in a frozen condition at a temperature varying from -15° to -8° C.,

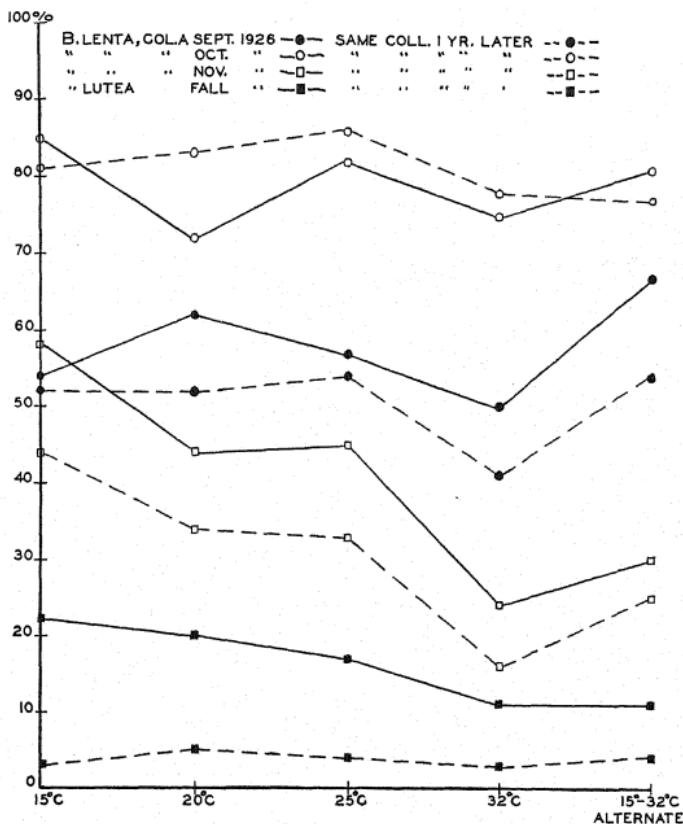


FIG. 3.—Germination of *Betula lutea* and different collections of *B. lenta* seeds at various temperatures shortly after harvest and one year after harvest; all samples after-ripened for six weeks at 8° C. before being transferred to germination chamber.

but since germination tests showed that most of the seeds stored at ice box temperature had not lost in vitality, this last set of samples was left unopened to be tested after a storage period of two years or more. The data in table XV show clearly that seeds of *Betula lenta*,

stored with a moisture content of 8.2 per cent or lower, keep perfectly well during the first year of storage at room temperature. With

TABLE XIV

AMOUNT OF HYGROSCOPIC MOISTURE IN *BETULA* SEEDS IN EQUILIBRIUM WITH VARIOUS CONCENTRATIONS OF H_2SO_4 AND WITH QUICKLIME

| DRYING AGENT | RELATIVE HUMIDITY OVER SOLUTION AT ROOM TEMPERATURE | HYGROSCOPIC MOISTURE IN PERCENTAGE DRY WEIGHT | | |
|------------------------------|---|---|--|---|
| | | <i>B. lenta</i> , October | <i>B. papyrifera</i> | <i>B. populifolia</i> |
| CaO..... | 0 | 0.01 | 0.6 | 0.4 |
| Concentrated H_2SO_4 | 0 | 0.6 | 0.7 | 0.1 |
| 64.8% H_2SO_4 | 10 | 4.4 | 4.8 | 5.2 |
| 55.9% H_2SO_4 | 25 | 6.3 | 6.4 | 7.2 |
| 43.4% H_2SO_4 | 50 | (original moisture content 7.09) 8.2 | 8.7 (original moisture content 8.7) | 8.5 (original moisture content 7.36) |
| 30.4% H_2SO_4 | 75 | 11.8 | 11.9 | 11.5 |
| 18.5% H_2SO_4 | 90 | 17.6 | 17.8 | 17.8 |

TABLE XV

GERMINATION OF *BETULA LENTA* SEEDS, COLLECTION OCTOBER, 1926, SEEDS OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE BOX; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

| DRYING AGENT | MOIS-TURE CON-TENT | PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE | | | | PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST | | | |
|------------------------------|--------------------|--|--------|--------|----------------------------|---|--------|--------|----------------------------|
| | | 15° C. | 20° C. | 32° C. | Alter-nation of 15°-32° C. | 15° C. | 20° C. | 32° C. | Alter-nation of 15°-32° C. |
| CaO..... | 0.01 | 0 | 2 | 62.5 | 80.5 | 0 | 4.5 | 38 | 74.5 |
| Concentrated H_2SO_4 | 0.6 | 0 | 4 | 62.5 | 73 | 0 | 6.5 | 47.5 | 74.5 |
| 64.8% H_2SO_4 | 4.4 | 0 | 2 | 53.5 | 84 | 0 | 2 | 50 | 79 |
| 55.9% H_2SO_4 | 6.3† | 0 | 0 | 57 | 68.5 | 0 | 2 | 69 | 84 |
| 43.4% H_2SO_4 | 8.2 | 0 | 1 | 32 | 76 | 0 | 0 | 43 | 74 |
| 30.4% H_2SO_4 | 11.8 | 0 | 0 | 0 | 0 | 0 | 0 | 70 | 77 |
| 18.5% H_2SO_4 | 17.6 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 5 |

* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. lenta*, collected October, 1926, 7.09.

a moisture content of 11.8 per cent seeds do not keep in room temperature, but retain part of their viability at 8° C. With 17.6 per cent

of moisture seeds lose their vitality in low temperature storage as well as at room temperature.

TABLE XVI

GERMINATION OF *BETULA POPULIFOLIA* SEEDS, COLLECTION OCTOBER, 1926, OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE CHEST; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

| DRYING AGENT | MOIS-TURE CONTENT | PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE | | | | PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST | | | |
|---|-------------------|--|--------|--------|----------------------------|---|--------|--------|----------------------------|
| | | 15° C. | 20° C. | 32° C. | Alter-nation of 15°-32° C. | 15° C. | 20° C. | 32° C. | Alter-nation of 15°-32° C. |
| CaO..... | 0.4 | 0 | 26.5 | 48.5 | 43 | 0 | 17.5 | 66.5 | 77.5 |
| Concentrated H ₂ SO ₄ | 0.1 | 0 | 23 | 46 | 36 | 0 | 8.5 | 49.5 | 44 |
| 64.8% H ₂ SO ₄ | 5.2 | 0 | 23 | 73 | 68 | 0 | 14.5 | 57.5 | 63.5 |
| 55.9% H ₂ SO ₄ | 7.2† | 0 | 38 | 63 | 47 | 0 | 37.5 | 82 | 64.5 |
| 43.4% H ₂ SO ₄ | 8.5 | 0 | 12 | 44 | 43 | 0 | 13 | 47.5 | 71.5 |
| 30.4% H ₂ SO ₄ *..... | 11.5 | 0 | 0 | 0 | 0 | 0 | 22.5 | 53.5 | 71.5 |
| 18.5% H ₂ SO ₄ *..... | 17.8 | 0 | 0 | 0 | 0 | 0 | 2 | 7.5 | 9.5 |

* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. populifolia*, 1926, 7.2.

TABLE XVII

GERMINATION OF *BETULA PAPYRIFERA* SEEDS OF VARIOUS MOISTURE CONTENTS STORED AIR-TIGHT FOR ONE AND ONE-HALF YEARS AT ROOM TEMPERATURE AND IN ICE CHEST; SEEDS NOT AFTER-RIPENED; 400 SEEDS IN EACH TEST

| DRYING AGENT | MOIS-TURE CONTENT | PERCENTAGE GERMINATION AFTER STORAGE AT ROOM TEMPERATURE | | | | PERCENTAGE GERMINATION AFTER STORAGE IN ICE CHEST | | | |
|---|-------------------|--|--------|--------|----------------------------|---|--------|--------|----------------------------|
| | | 15° C. | 20° C. | 32° C. | Alter-nation of 15°-32° C. | 15° C. | 20° C. | 32° C. | Alter-nation of 15°-32° C. |
| CaO..... | 0.6 | 0 | 51 | 64.5 | 69 | 1 | 35.5 | 70 | 77.5 |
| Concentrated H ₂ SO ₄ | 0.7 | 0 | 33 | 03.5 | 79 | 0 | 63 | 92.5 | 83.5 |
| 64.8% H ₂ SO ₄ | 4.8 | 0 | 12 | 32 | 49 | 0 | 37 | 77 | 77.5 |
| 55.9% H ₂ SO ₄ | 6.4 | 0 | 5 | 8 | 29 | 0 | 78.5 | 84 | 91 |
| 43.4% H ₂ SO ₄ | 8.7† | 0 | 3 | 7.5 | 11 | 0 | 41 | 81.5 | 64 |
| 30.4% H ₂ SO ₄ *..... | 11.9 | 0 | 0 | 0 | 0 | 0 | 26 | 82.5 | 80 |
| 18.5% H ₂ SO ₄ *..... | 17.8 | 0 | 0 | 0 | 0 | 0 | 4.5 | 6 | 6.5 |

* Seeds kept for entire storage period over solutions with higher vapor pressure than that of laboratory air.

† Original moisture content of *B. papyrifera*, commercial collection 1926, 8.7.

The behavior of *B. populifolia* is somewhat different from that of *B. lenta*, as is shown in table XVI. The keeping quality of *B. populi-*

folia was reduced by storage with a very low hygroscopic moisture, as well as by storage with a water content as high as that of freshly harvested seeds and higher. The optimum moisture content for storage at room temperature seems to be about 5.2 per cent. With seeds kept in the ice box, the importance of the water content decreases for *B. populifolia* in the same way that it does for *B. lenta*.

A third type of reaction is obtained when seeds of *B. papyrifera* are stored with different amounts of hygroscopic moisture (table XVII). Seeds stored at room temperature keep well with greatly reduced moisture content only, lose a considerable amount of vitality when stored with the original moisture content of freshly harvested seeds, and die completely when stored with increased water content.

The three types of keeping quality may be characterized briefly in the following way:

TYPE 1 (*B. lenta*).—At ordinary room temperature seeds keep well with a moisture content ranging from 0.01 to 8.2 per cent (m.c. at time of harvest 7.9 per cent). With a m.c. higher than 8.2 per cent seeds keep only when stored at ice chest temperature.

TYPE 2 (*B. populifolia*).—At ordinary room temperature seeds keep well only at the slightly reduced moisture content of about 5.2 per cent (m.c. at time of harvest 7.2 per cent). With a m.c. reduced below or increased above 5.2 per cent seeds keep well only at ice chest temperature.

TYPE 3 (*B. papyrifera*).—At ordinary room temperature seeds keep well only with a highly reduced moisture content of 0.6 or 0.7 per cent (m.c. at time of harvest 8.7 per cent). With a m.c. above 0.7 per cent seeds keep well only at ice chest temperature.

At a moisture content of about 17.6 per cent all three types lose their vitality completely at room temperature, and almost completely at ice chest temperature, within the first year of storage. A comparison of germination obtained at the optimum germination temperature of 15° – 32° C. alternation in all three species of *Betula* stored at various moisture contents in room temperature is given in the curves shown in fig. 4. Fig. 5 shows the modifying influence of a low storage temperature under otherwise similar conditions.

It is of interest to note that although seeds containing a high amount of hygroscopic moisture keep comparatively well at a cool

storage temperature, they do not after-ripen, as is shown by the fact that no germination is obtained at 15°C . as is characteristic for

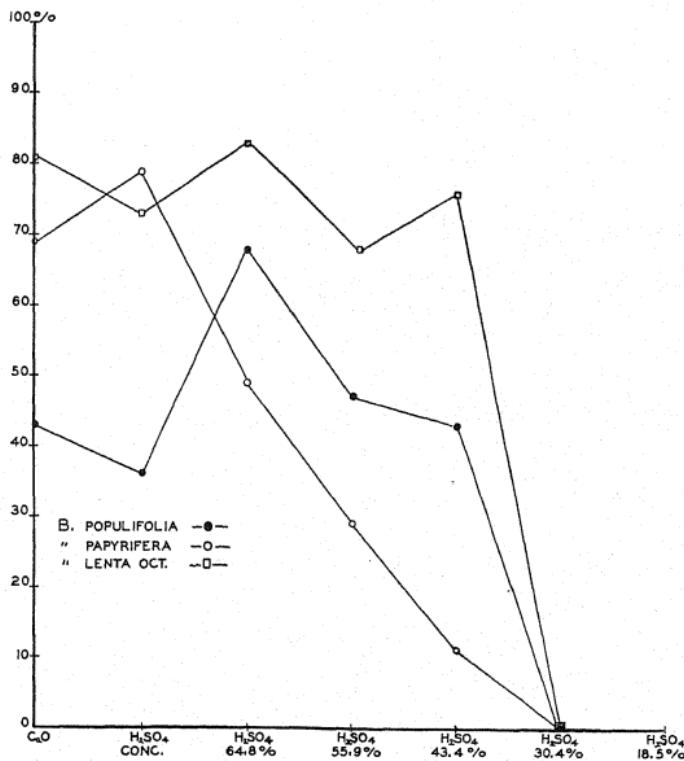


FIG. 4.—Germination of *Betula* seeds after one year of storage at room temperature in hermetically sealed vessels in atmospheres of different humidities; germination at alternating temperatures, $15^{\circ}-32^{\circ}\text{C}$. (eighteen hours a day at 15° and six hours a day at 32°C .).

after-ripened seeds. To secure after-ripening at low temperatures a much higher moisture content of the seeds is required.

Discussion

In a discussion of the results obtained in these experiments, three facts call for special attention: (1) the behavior of freshly harvested

seeds as compared with laboratory-dry seeds; (2) the effect of moist cool storage upon the temperature requirements of germination; and

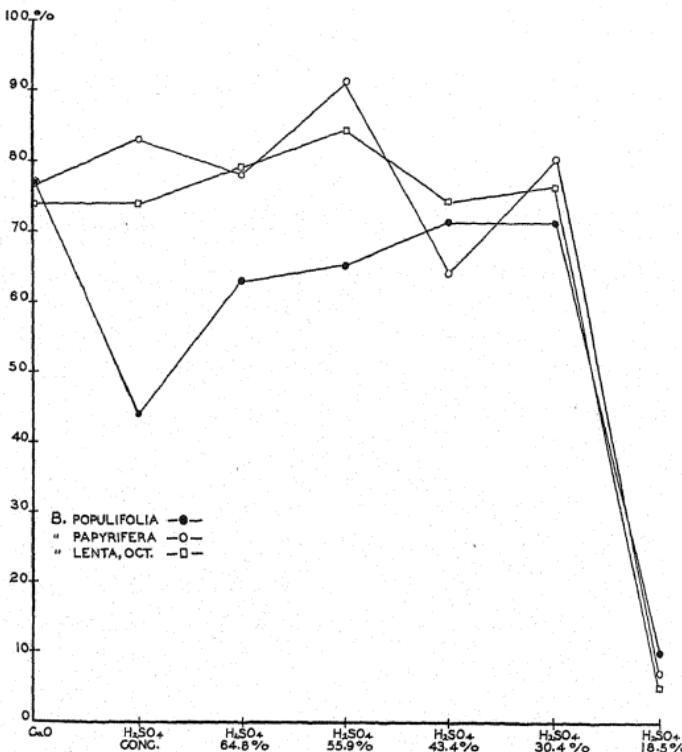


FIG. 5.—Germination of *Betula* seeds after one year of storage in ice chest in hermetically sealed vessels in atmospheres of different humidities; germination at alternating temperatures, 15°–32° C. (eighteen hours a day at 15° and six hours a day at 32° C.).

(3) the relation of moisture content to keeping quality in the different species studied.

It has been shown that fresh seeds of *B. lenta* germinate very poorly immediately after harvest, but improve considerably in germination quality during one month of dry storage. Causes for this improvement may be due to internal changes as well as to an

alteration in seed coat characters. If the changes are internal, they must be different from those that could be produced through variations in moisture content in the seed, or through the influence of frost and other weather conditions while the seeds are still attached to the tree, since different samples of seeds harvested at different times of the season and containing various amounts of hygroscopic moisture were all improved by one month of dry storage. Changes in the seed coat, such as an increase in permeability to water, oxygen, or carbon dioxide produced by a continuous drying at temperatures higher than those prevailing out-of-doors during the harvesting season, may also be responsible for the beneficial effect of dry storage. Unfortunately the small size of birch seeds and the tightness with which the coat fits around the embryo made it impossible to remove the coat of fresh seeds successfully in order to find out whether or not the fresh coat was responsible for the failure of the seeds to germinate.

Many seeds require low temperature stratification to prepare them for germination. This is true of rosaceous seeds (1, 2, 5, 6, and 7), *Tilia* (11), *Juniperus* (10), *Acer* (8), *Cornus*, *Sambucus*, and *Berberis* (3, 4). In all of these high temperatures are ineffective in forcing germination previous to after-ripening in low temperature stratification. *Betula* species differ from this type in so far as they need a period of cool storage only to enable the seed to germinate at the lower temperatures, as 15° and 20° C. At temperatures such as 32° C. and an alternation of 15°–32° C. the seeds germinate without previous treatment. When the samples are kept moist at after-ripening temperatures, such as 0°, 5°, or 10° C. for longer periods than those recorded in this paper, the seeds finally begin to germinate at the storage temperatures, those stored at 10° growing first, and those stored at 0° C. beginning to grow only after 5–6 months.

In this study it was found that the species which required cool storage for later germination at the lower temperatures proved most resistant when stored under various conditions, while the less "dormant" species, *B. papyrifera*, lost its vitality much easier under unfavorable conditions of storage. A physiological or chemical basis for the relation of keeping quality to dormancy has not been worked out.

Conclusion

I. GERMINATION

1. Freshly harvested seeds of *Betula lenta* germinate poorly regardless of time of harvest, moisture content, and dry weight of the seeds at harvest time. The germination quality improves considerably during the first month of dry storage at laboratory temperature.

2. The optimum germination temperature for air-dried seeds of *B. lenta*, *B. papyrifera*, *B. lutea*, and *B. populifolia* is 32° C. constant, or alternation between 15° and 32° C.

3. The minimum germination temperature for dry-stored seeds is remarkably high, being about 30° for *B. lenta*, and about 20° C. for *B. populifolia*, *B. papyrifera*, and *B. lutea*. The dry-stored seeds have a narrow temperature range for germination.

4. When air-dry or freshly harvested seeds of *Betula* are kept in an imbibed condition at low temperatures they after-ripen, or go through a series of changes that improves their germination at high temperatures and enables them to germinate at much lower temperatures. The most favorable temperatures for after-ripening are 0° to 5° C., while 10° is less favorable, and storage in a frozen condition is ineffective or even injurious. Four weeks of stratification at 0° or 5° puts the seeds into condition for excellent germination at 15° C. Six to eight weeks of stratification at these temperatures reduces the minimum germination temperature sufficiently to give good early spring germination in outdoor seed beds. Seeds stratified at 0° C. for 5-6 months begin to germinate profusely even at this low temperature. In short, the minimum germination temperature falls 20°-30° C. with such treatment.

5. Moist sand, granulated peat, or blotting or filter paper are equally effective as stratification media, so that the seeds are indifferent to a considerable range of acidity in the medium.

6. Seeds of *B. papyrifera* are less dormant than are those of the other species studied. They also fall in vitality most rapidly in unfavorable storage condition.

7. The falling of the germination minimum with low temperature stratification must be advantageous to the seeds in nature. The high germination minimum of the freshly shed or dry seeds will insure no germination in the fall. The cold weather of the winter, with the

seeds buried under leaves and snow, will after-ripen them and prepare them for early spring germination. This probably accounts for the abundance of *Betula* seedlings in the early spring. Knowledge of the after-ripening at low temperature stratification is also very important to the producer of birches. Dry-stored seeds sown outside in early spring will not germinate because of the high temperature minimum, while properly stratified seeds will give quick and abundant germination in early spring.

8. The percentage germination of *Betula* seeds reported in this paper is very much higher than that reported in forestry books (12). This is probably easily explained by the facts that past workers have used too low temperatures for the germination of dry-stored seeds, or they have failed properly to after-ripen the seeds that are to be germinated at low temperatures in the seed beds in the early spring.

9. Germination of dry-stored *Betula* seeds is not affected by a considerable range of acidity of the medium in which germination takes place; neither is it influenced by light, increased CO_2 , or a considerable range of O_2 pressure. Sterilization with 0.2 per cent uspulon for one-half hour causes a slight reduction in percentage of germination.

II. STORAGE

10. During one year of air-dry storage at room temperature, seeds of *B. lenta* and *B. populifolia* kept perfectly, while *B. lutea* and *B. papyrifera* fell in viability.

11. The optimum moisture content for seeds stored at room temperature in sealed containers lies considerably below the moisture content of freshly harvested seeds for *B. papyrifera* (0.6 per cent), while *B. populifolia* keeps best with a medium amount of hygroscopic moisture (5.2 per cent). *B. lenta* keeps well in all except very humid conditions during a storage period of one year.

12. Stored at ice box temperature, seeds with higher moisture content keep as well as seeds low in hygroscopic moisture for one and one-half years of storage.

III. SUGGESTIONS FOR GROWERS

13. Birch seeds are harvested to best advantage after the catkins have begun to dry on the trees, but before they have shed a con-

siderable part of the seeds. They should be shaken from the catkins, dried on well ventilated racks, and stored. About six weeks before planting the seeds ought to be stratified in a suitable moist medium at temperatures of from 32° to 41° F. After such a treatment the seeds will germinate in the seed beds in early spring. It takes approximately one month to six weeks for a full stand of young seedlings to appear above the surface of the soil. Early spring planting is recommended.

14. For dry storage of seeds for a few months no special precautions need be taken, but for storage of one year or more the following methods ought to be used: *B. lenta* can be stored in almost any storage room of average room temperature that is dry and well ventilated; *B. populifolia* is sensitive to excessive drying as well as to very high humidities, and should therefore be kept in closed containers at a cool temperature; *B. papyrifera* keeps best when thoroughly dry, which can be done by keeping the seeds suspended in a bag in a closed container, the bottom of which is covered with quick lime.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

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AN INEXPENSIVE AND QUICKLY MADE INSTRUMENT FOR TESTING RELATIVE HUMIDITY¹

WILLIAM B. SHIPPY

(WITH THREE FIGURES)

It is commonly understood that sulphuric acid and various salts may be used in the control of humidity. WILSON² discusses the use of sulphuric acid in humidity control, and SPENCER³ presents a list of inorganic salts supplying a rather wide range of humidities.

Most investigators attempting to control humidity do not actually make humidity readings. The usual procedure is to check the specific gravities of the control solutions from time to time. It is taken for granted that a given concentration of solution will give a specified vapor pressure, but such an assumption may lead to error. To illustrate: A given concentration of sulphuric acid solution under specific temperature and pressure conditions will exert a definite humidity control through desiccation of a limited volume of air. Any modification of these prescribed conditions will necessarily result in a humidity variation of the air under control; hence, any decrease or increase in temperature brings about a corresponding change in vapor pressure. In addition to the preceding, there is often the possibility of leakage in tubing, ill-fitting stoppers, or defective glassware. If living plant material is placed within the controlled vessel, large quantities of water will be given off through transpiration. A further source of difficulty lies with inaccuracies due to the control solution itself, which may contain impurities causing it to give a higher specific gravity reading than would correspond to its sulphuric acid content. Defective recording instruments also might

¹ This work was done in connection with a study sponsored jointly by the Boyce Thompson Institute for Plant Research, Inc., Yonkers, New York, and the Crop Protection Institute, and published at the expense of the former Institute out of the order determined by the date of receipt of the manuscript.

² WILSON, R. E., Humidity control by means of sulfuric acid solutions, with critical compilation of vapor pressure data. *Jour. Indus. and Engin. Chem.* 13:326-331. 1921.

³ SPENCER, H. M., Laboratory methods for maintaining constant humidity. *Internat. Critical Tables* 1:67-68. 1926.

mislead one. In the last analysis it is not the potential desiccation power of the control solution that really matters; it is the humidity actually present within the controlled vessel, and this can only be determined by measurement. Thus it is apparent that difficulties lie in the pathway of any one attempting accurate humidity control. Specific gravity determinations of a control solution are not sufficient evidence under ordinary experimental conditions that a definite vapor pressure has been attained.

Various instruments are available for humidity testing, including the dew-point apparatus, instruments of the wet and dry bulb principle, and various types of hair hygrometers. These vary in reliability, but among the best are those of the wet and dry bulb type.

The writer began studies requiring constant humidities during the spring of 1927. It was found desirable to use an arrangement providing for a continuous flow of air through the controlled vessels, and a need arose for some instrument which would check the humidities delivered by the control solutions. The instrument here described, employing the principle of the wet and dry bulb, was designed to fill this need. It is illustrated in figs. 1-3.

An ordinary $3\frac{1}{4}$ -inch pyrex test tube (fig. 1D) was drawn out at the bottom and joined with a $1\frac{1}{4}$ -inch neck of quarter-inch glass tubing (H). The test tube was fitted with a three-hole rubber stopper (C). Two 100° F. matched thermometers (A) and an elbow of quarter-inch glass tubing (B) were inserted through the stopper. The thermometers were so arranged that when the tube was stoppered, the dry bulb would be approximately three inches and the wet bulb (carefully wrapped with one thickness of clean muslin, which had been thoroughly soaked in water) four inches from the base of the tube. For convenience in holding the instrument, it was commonly placed within an empty suction flask (fig. 3N).

Air forced by compression (applied at M) or drawn by vacuum (applied at O) from the control solutions (P) through this test tube past the bulbs from the bottom inlet (H) to the top outlet (B) gives a reading within a few minutes, the time depending on the rate of air flow. The rate of air flow does not call for great precision, but uniformity of flow is desirable. A flow of one liter per minute (moderately rapid bubbling) is a good rate to use in

Fig. 1.

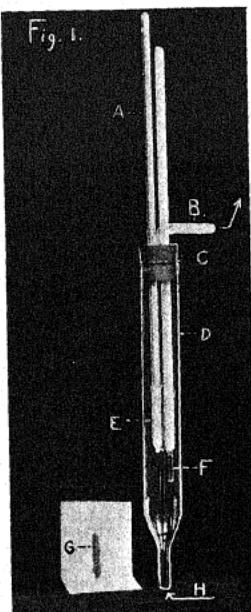


Fig. 2.

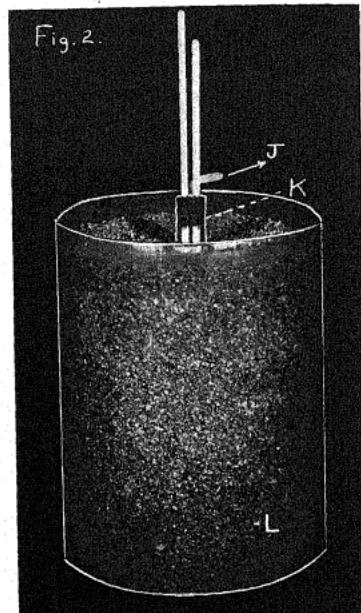
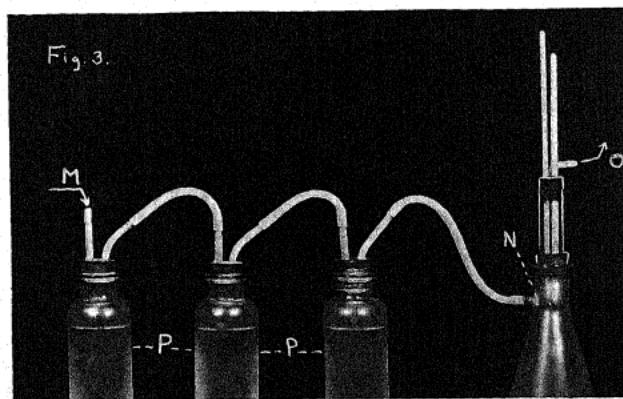


Fig. 3.



FIGS. 1-3.—Fig. 1, instrument for testing humidity: A, two matched thermometers; B, air outlet tube to which vacuum tube is attached; C, 3-hole rubber stopper; D, pyrex test tube; E, wet bulb; F, dry bulb; G, fine-mesh copper screen plug; H, air inlet tube. Fig. 2, testing relative humidity within solid medium: J, point at which vacuum tube is attached; K, approximate depth instrument is buried in medium; L, peat moss medium. Fig. 3, testing relative humidity of air passing through control solution: M, air inlet where compressed air tube may be attached; N, empty suction flask used to hold instrument; O, air outlet where vacuum tube may be stored; P, control solution.

checking most solutions, but satisfactory readings may be made over a fairly wide range. In testing a solution of low vapor pressure, it is not desirable to use too slow an air flow, as the cloth on the wet

TABLE I

TYPICAL SET OF READINGS WITH INSTRUMENT COMPARED WITH THEORETICAL RELATIVE HUMIDITIES AS INDICATED BY SPECIFIC GRAVITIES OF CONTROL SOLUTIONS
OCTOBER 18, 1928; RATE OF AIR FLOW ONE LITER PER MINUTE

| DETERMINATIONS BY INSTRUMENT | | | | DETERMINATIONS BY SPECIFIC GRAVITY | | |
|-------------------------------|----------|------------------------|------------------|---|--------------------------------------|--|
| Readings | | Depression of wet bulb | Room temperature | Relative humidity ⁴ (per cent) | Specific gravity of control solution | Corresponding relative humidity ⁵ |
| Wet bulb | Dry bulb | | | | | |
| Solution I (water) | | | | | | |
| 74.5* | 75.5* | | | | 1.000 | 100 |
| 76.75† | 77 † | 0.25 | 77 | 99 | | |
| 75.5 | 77 | | | | | |
| 77.25 | 77.5 | 0.25 | 77 | 99 | 1.000 | 100 |
| Solution II (sulphuric acid) | | | | | | |
| 78 | 80 | | | | 1.190 | 81 |
| 73.5 | 79 | 5.5 | 79 | 78 | | |
| 75. | 79 | | | | | |
| 74 | 78.75 | 4.75 | 79 | 81 | 1.190 | 81 |
| 77 | 77.5 | | | | | |
| 73.5 | 78.25 | 4.75 | 78 | 81 | 1.190 | 81 |
| Solution III (sulphuric acid) | | | | | | |
| 79 | 80 | | | | 1.265 | 65 |
| 71.5 | 80 | 8.5 | 80 | 68 | | |
| 75 | 80 | | | | | |
| 71.5 | 80 | 8.5 | 80 | 68 | 1.265 | 65 |
| 75 | 79 | | | | | |
| 70 | 78 | 8.0 | 78 | 69 | 1.265 | 65 |

* Initial readings.

† Final readings.

⁴ MARVIN, C. F., Psychrometric tables for obtaining the vapor pressure, relative humidity, and temperature of the dew-point. U.S. Weather Bureau, no. 235. 78-79. 1915.

⁵ WILSON, R. E., Graph on page 328 (article cited).

bulb may dry before time has been allowed for the reading. The reading is made when both mercury columns have come to rest. This makes possible a quick and satisfactory check on the humidity delivered by a control solution. To illustrate how the tester is used, a typical set of determinations on three solutions is shown in table I.

The instrument described can be used for testing relative humidity within solid media. If the investigator desires to know the humidity within a solid medium, as peat moss or a fairly open soil, the basal neck of the tube (H) may be fitted with a plug of fine-mesh copper screen (fig. 1G) to prevent solid particles from entering. The instrument is forced down into the medium (fig. 2L) almost to the top (K), the vacuum tube applied (at J) and a reading made. Such tests are useful in work on the stratification of seeds or in the storage of cuttings, root-grafts, or other similar materials. It may be said in this connection that there is danger of inaccuracy if small volumes of medium are tested, as the replacement of air between the medium particles from the outside would be too great.

One may test with this instrument the relative humidity of large or small volumes of air, 5-10 liters being sufficient for a reading. The size and shape of the instrument make possible its insertion through small openings, such as the hole in the lid of a desiccator or the top of a bell jar. Thus readings can readily be made out of doors or within constant condition chambers of large or small size. In addition to a rather wide use as a tester of humidity, it has the dependability of the sling psychrometer. It may be quickly constructed in any laboratory and is made of standard equipment in common use.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

RÔLE OF MOTHER TUBER IN GROWTH OF POTATO PLANT¹

F. E. DENNY

(WITH FIVE FIGURES)

Introduction

Substances stored in the mother tuber are utilized by the sprout as soon as germination begins, and many experiments have shown that the sturdiness of the sprout, the subsequent rate of growth, and the final yield are related to the size of the seed-piece at the time of planting. Questions relating, however, to the stage of development at which the sprout becomes entirely independent of the mother tuber, the nature of the substances passing to the sprout which are so influential in modifying its growth, the rate at which different compounds pass from the tuber to the sprout, and the correlation in point of time which may exist between this transfer and the subsequent growth of the plant, these questions either find no answer in the literature of the subject or the answers that have been provided are conflicting.

By a series of experiments carried out in 1926, 1927, and 1928, it is believed that additional information upon these points has been obtained. The method of study has been, in brief, to amputate the mother tuber (by a method which involved a minimum disturbance of the root system) at different intervals after planting, that is, at different stages in the development of the plant, to observe the effects of this amputation upon the subsequent growth and final yield by comparison with check plants from which mother tubers had not been removed, to determine by chemical analyses of the amputated mother tissue what substances had disappeared and at what rate, and finally to make use of these observations to determine the periods at which connection with the mother tuber is critical,

¹ Contributions from the Boyce Thompson Institute for Plant Research, Inc., Yonkers, N.Y., published at the expense of the Institute out of the order determined by the date of receipt of the manuscript.

and to correlate the chemical composition at any period with behavior after amputation.

In these experiments an effort was made to deal with sufficiently large numbers of plants so that the influence of experimental errors would be reduced. During the three years, about 3000 plants, including amputated and checks, have been used in the tests. Consequently the samples of tissue taken for analyses have represented large numbers of individual tubers, and, since the yield data were obtained separately for each plant, the influence of variability (which is high in experiments on potato yield) could be measured and allowed for. Furthermore, although only two varieties, Irish Cobbler and Bliss Triumph, were used in the main experiments, preliminary tests were also made with ten other varieties, and it is likely that the results can be considered applicable to a considerable number of varieties. In one respect these experiments have been narrowly restricted, however, in that in nearly all cases only one size of seed-piece has been used, namely, 28 gm. The influence of size of seed-pieces upon these results requires further experimentation.

Summary of literature

The attempt is not made here to deal chronologically with the various papers that have been published on this and related subjects, but rather to indicate the relation which they bear to the particular phases concerned in the present experiments, and to show the status of the subject at the time the work was initiated.

Amputation experiments were carried out by SIKORSKI (18), who on May 20 planted six potato tubers (weight 63–66 gm.), three in soil and three in sand. He removed the mother tuber from one plant in each series on June 30, and from another plant on August 31. As compared with plants from which mother tubers were not removed, it was found that amputation had reduced the yield in all cases. He believed that the influence of the mother tuber is not restricted to the early stage of development, but extends over the complete vegetative period. He suggested that in the later stages the mother tuber acts as a storage organ for water, and that this function is very important in periods of drought.

More recently SELIBER (16, 17) has furnished two reports of his

amputation experiments, in which larger numbers of plants were dealt with. His method of removing the mother tuber from the growing plant was to undermine the plant by removing earth until the mother tuber could be removed by hand. Two types of check plants were used, one being plants that were undermined in the same way until the mother tuber was touched by the hand but was not removed, and the other being plants which grew continuously in the soil without disturbance. He found that the removal of the mother tuber, even in midseason, reduced the final yield; but that the variety of potato was an important factor, the varieties Reitan and Chugunka being influenced to a great extent by amputating the mother tuber, while in the varieties Kruger and Epicurez the substance of the mother tuber was not utilized. With these latter varieties, presence or removal of the mother tuber was not a matter of importance, so far as the yield was concerned. SELIBER also discusses the importance of the mother tuber as a water storage organ.

The literature relating to the extent to which the substance of the mother tuber is utilized, that is, as to the amount of reduction in dry weight during the growing season, is conflicting. The earlier workers were impressed by the large proportion of dry substance that is removed from the mother tuber, while certain more recent reports tend to show that considerable amounts of the stored foods remain in the seed-piece even at the end of the growth period.

As an example of the first group, we may cite FITTBØGEN, GROENLAND, and FRAUDE (9), who carried out the first and most complete set of analyses of mother tubers at intervals during the growth of the plant. They planted the tubers on April 13 and dug up sample lots of plants on May 28, June 18, July 2, July 25, August 20, and September 22. The analyses of the mother tubers on these dates showed a rapid decrease in protein, starch, fat, "undetermined organic," and ash. More than 70 per cent of the organic substance of the mother tuber had been lost by June 18.²

MÜLLER (12) noted that even at the end of a dry summer the mother tubers that were recovered from potato vines at harvest were not shrunken, but were full of sap. But his analyses of the recovered

² Their analyses show that in spite of this loss of dry matter, the sugar content of the mother tubers increased, and the present experiments confirm this finding.

seed-pieces showed that the dry substance represented about 3.7 per cent of the fresh weight, that almost all of the starch, protein, and phosphorus compounds had disappeared, and that ash constituents (except KCl) had been reduced to about one-half the original amount.³

In one of the more recent articles relating to the utilization of the stored food, JONES and ROSA (10) state:

The analyses of RAMSEY and ROBINSON (1917) show that carbohydrate, N, and ash constituents of the mother set are far from exhausted, even at the end of the growing season.

Still more recently we have the report of LUDWIG (11), which contains data on the composition of the mother tuber after growth in culture solutions. His experiments showed that after the sprouts had become 6, 8, or even 30 cm. high, with normal dark green leaves, and with roots and stolons 20 cm. long, the mother tubers still retained 40 per cent of their total nitrogen and about 65 per cent of their carbohydrate reserves. He emphasizes the "common observation" that eyes dug out of tubers will produce normal plants with tubers not inferior to those produced by seed tubers planted in the usual way. He states that, during the late war, farmers used not only pieces of tubers, but also merely well developed eyes attached to pieces of potato peelings, and these gave normal yields. The failures that had resulted from the use of cut tubers were thought to be due, not to the small quantity of reserve food in the seed-pieces, but to loss by rotting.

DE VRIES (6) studied the transfer of food reserves from the mother tuber to the sprout at different stages of growth. These observations were largely qualitative, no analyses being furnished and no amputation experiments being carried out. His opinion was that the mother tuber furnished nutrients to the sprout until germination was completed (plants above ground with green leaves), but that after the beginning of formation of young stolons the food supply was thenceforth transferred only to the newly forming tubers.

³ One paragraph in MÜLLER'S article is significant in connection with the present experiments. He states: "It would be interesting to carry out a special experiment to determine in what manner the absolute weight of the mother tuber changes during the vegetative period. Also, how the yield of young tubers would be influenced by removing the mother tuber after the sprout had completely developed."

Although, as will be shown later, the evidence is in favor of the older view that the depletion of the food reserves stored in the mother tuber is extensive or nearly complete, there is no agreement as to the nature of the substances furnished by the mother tuber that are so influential in increasing growth. APPLEMAN (1), by a series of experiments on the relation between the size of the seed-pieces and the strength of the sprout, showed that when the size of the seed-piece fell below a certain minimum, the vigor of the sprout decreased as the size of the seed-piece was reduced. He believed that the failure of small pieces to produce good sprouts was not due primarily to the lack of usual food materials, "as sprouts on pieces still large enough to contain an abundance of these substances show considerable decrease in vigor." He concluded "that the potato tuber contains a limited amount of a special growth promoting substance, and if the amount of tissue surrounding the growing bud is too small there is not enough of this substance available for normal growth." The view expressed by JONES and ROSA (10) is that "the large set furnishes something to the plant arising from it, which is not present in sufficient amount in the smaller set."

From this short summary of the literature, it is apparent that further information is needed upon the subject. In previous amputation experiments either not enough plants have been used, or not enough stages of development of the plants have been included in the test to give a complete account of the relation of the mother tuber to the sprout. The observations on the effect of the removal of mother tubers have not been accompanied by chemical analyses of the amputated tissue. The data as to the extent of the utilization of the mother tuber show sharp disagreements, and further analyses are needed to show which of these divergent views is correct. The present experiments have provided additional information upon several of the questions involved, but a discussion as to the bearing of the results upon various phases of the problem will be postponed until the experimental results themselves have been described.

Results

The experimental results may be divided into two parts, the first dealing with the amputation of the mother tubers at intervals after planting and its effect upon subsequent growth of the plant, the

second dealing with the chemical composition of the mother tissue that was removed at different periods of growth.

AMPUTATION OF MOTHER TUBER AND ITS EFFECT

METHOD OF REMOVAL FROM SPROUT.—Since it was essential that the mother tuber be removed from the sprout with as little disturb-

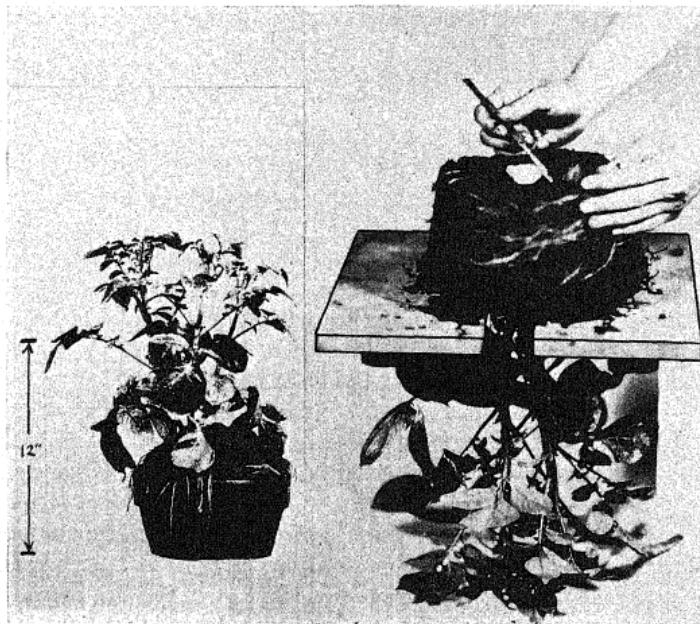


FIG. 1.—Method of amputating mother tuber from sprout: left, plant grown in pot buried in soil; right, plant inverted, placed upon board with slot from edge to center used for support; pot then discarded. Note mother tissue being removed piece by piece with scalpel; plant placed in soil after removal of mother tuber.

ance of the root system as possible, the plants were grown in clay pots which were planted in the soil, the top of the pot being slightly below the surface of the soil. At any subsequent period of growth at which it was desired that the mother tuber be removed, a number of pots containing the now well-rooted plants were removed from the soil and inverted (fig. 1). The mother tuber, which had been

planted in the bottom of the pot just above the drainage hole, was now freely exposed and could easily be detached by cutting; the pot was then discarded and the plant was replaced in the soil, and from this time onward grew under normal conditions, that is, undisturbed by the restrictions of growth in pots.

At each stage of development at which mother tubers were removed from a certain number of plants, an equal number of plants were removed from pots and planted in the soil, but from these the mother tubers were not removed. These were considered as check plants, since they were handled in precisely the same way except with reference to the removal of mother tubers. It is true that there would be somewhat greater disturbance of the soil in removing the mother tubers than would result in handling the checks, but an attempt was made to equalize this difference partially, by purposely disturbing the soil around the mother tubers in the check to about the same degree.

In setting the plants in the soil, the arrangement of the amputated plants and their corresponding checks depended upon the stage of development at which the mother tubers were removed. When they were amputated at early stages, for example, at stages 1 and 2 (fig. 2), the plants were arranged in the field in sets containing five plants each, that is, five plants of the amputated series, then five plants of the check series, then five plants of the amputated series, etc. At the later stages of growth, for example, at stages 3 and 4 (fig. 2), however, the amputated plant and the corresponding check were planted side by side, in order to equalize soil differences as nearly as possible and to permit more dependable comparisons.

It was found that the plan of having a set of checks for each set of plants from which mother tubers were removed, and repeating this at each stage of growth at which they were removed, was essential in order to permit dependable comparisons at the end of the season. In the first year of the experiments (1926) this precaution was not taken, the "checks" being merely plants which had never been in pots at any part of the season and from which mother tubers were not removed. The yield data at the end of the season did not therefore take into account the influence of the growth in pots, as compared with growth in free soil, up to the time of removal of

mother tubers. Consequently the yield data for 1926 had to be rejected. In 1927 and 1928, however, the amputated lots and the corresponding check lots grew for the same periods in the same sized pots, and for the same periods in the soil, the only variable factor being presence and absence of the mother tuber.



FIG. 2.—Conditions of plants at four different stages at which mother tubers were amputated: *A*, stage 1; *B*, stage 2; *C*, stage 3; *D*, stage 4.

The preliminary experiments in 1926 also showed that the exact method of removing the mother tuber from the plant was a matter of considerable importance. When it was removed by cutting across the stem at the surface of the tuber, or by forcibly twisting it off from the stem, bleeding at the cut end of the stem occurred. This often resulted in wilting of the plant, even when the well-rooted plant with undisturbed root system was placed at once in soil to

which an abundant amount of water had been added. This difficulty was obviated in 1927 and 1928 by sectioning off small portions of tissue from the mother tuber piece by piece, until finally a small piece of it was allowed to remain at the base of the stem. Under these conditions no wilting was observed. The method of removing the mother tuber is shown in fig. 1, and the condition at the base of the stem after amputation was completed is shown in fig. 4 (see left-hand plant at K).

SIZE OF SEED-PIECE AND VARIETIES.—Seed-pieces weighing 28 gm. each were used, and in order to be assured of uniformity in this respect the pieces were weighed individually, and trimmed until the weight did not vary by more than 1 gm. In the 1926 and 1927 experiments only Irish Cobbler variety was used, but in the 1928 work Bliss Triumph was included in the main experiment. In this year also, a preliminary test was made with a number of other varieties, as shown in table VI, in order to note the behavior of various varieties with respect to utilization of the dry matter in the mother tuber.

SIZE OF POTS.—For the series in which the mother tubers were to be removed at stage 1 (fig. 2), clay pots 3.5 inches in diameter and 3.5 inches deep were used; for the series at stage 2 the pots were 6 inches by 4.75 inches; for the series at stage 3 they were 8 inches by 4.5 inches; and for stage 4 two sizes were used, 10 inches by 4.5 inches and 12 inches by 4.5 inches.

STAGES OF DEVELOPMENT AT WHICH MOTHER TUBERS WERE REMOVED.—The mother tubers were removed at four different stages of development, and the sizes of the plants at each amputation stage are shown in fig. 2. At stage 1 (A) the mother tubers were removed when the sprouts had just pushed through the soil. The tips were green, but the young leaves had not yet expanded. This was the condition 22 days after planting. At stage 2 (B) the mother tubers were removed when the sprouts were about 2 inches above the surface of the soil and the leaves fully expanded. This was the condition of the plants 29 days after planting. At stage 3 (C) the plants were about 10 inches above the surface of the soil, were making a vigorous growth, underground stolons several inches long had formed, and young tubers were well started in development. These plants might be termed half-grown. This was the condition 42 days after planting.

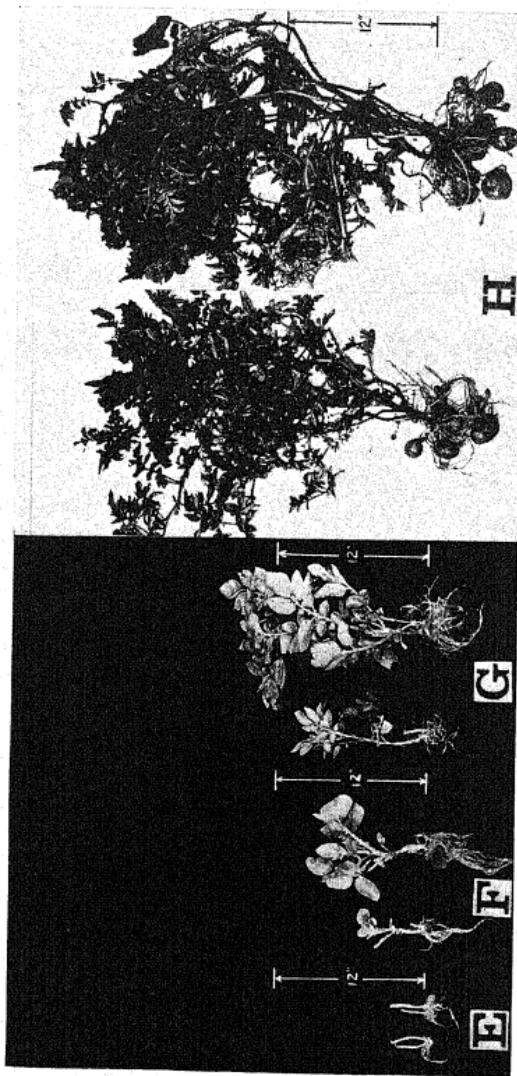


FIG. 3.—History of development of amputated and check plants during growth period when mother tuber was removed at stage 1: *E*, amputated (left) and check (right) at time of amputation, May 24; *F*, two plants of same series showing condition of amputated and check plants on June 8; *G*, further stage in growth of amputated (left) and check (right) plants as shown by representative plants on June 19; *H*, comparison of amputated (left) and check (right) plants on July 31.

At stage 4 (D) the plants were about 24 inches above the surface of the soil, had attained approximately the maximum height of vine, were in full bloom, and many young tubers were fully formed. This was the condition 56 days after planting.

SUBSEQUENT GROWTH OF PLANTS AFTER AMPUTATION.—The growth of the plants from which the mother tubers were removed as compared with the growth of the corresponding check plants is shown in figs. 3 and 4. These photographs were obtained by selecting what appeared to be average plants from each group, and placing the treated and check plant side by side for photographing. Because of the great variability of potato plants it will be recognized that only rough comparisons can be obtained in this way, since a single plant cannot be depended upon to show accurately the average condition of a group.

The subsequent growth of the plants from which mother tubers were removed at stage 1 is shown in fig. 3. The condition of the amputated plant and its check on the day of the removal of the mother tuber (May 24) is shown at E; and F, G, and H show the conditions of the plants on June 8, June 19, and July 31 respectively. It will be observed that the plants from which the mother tubers were removed made slow growth at first, but that later in the season a surprisingly large amount of vine was developed. The amputated plant shown in fig. 3 (H, left) shows a comparatively larger quantity of tubers, however, than is justified by the final yield data shown in table IB, thus indicating that although the plant used for the photograph showed an average condition of vine, the underground condition was not truly representative of the group. At any rate it is apparent that removing the mother tubers at this early stage influenced both the subsequent vine growth and tuber yield, and that the effect was greater upon the yield of tubers than upon the final size of vine.

The subsequent growth of the plants from which mother tubers were removed at stage 2 is shown in fig. 4. The condition of the amputated plant and its check (May 31) is shown at K, and the condition on June 19 is shown at L. From this date onward the amputated plants grew rapidly enough to overtake the checks, so that later in the season no difference in size of vine could be observed between amputated and check plants, and consequently no further photo-

graph in this series was made. As for the plants at stages 3 and 4, the condition of the checks is shown in fig. 2, C and D. The subsequent rates of growth of the vines by amputated and check plants in these two series were so nearly equal that no difference was observable and further photographs were not made. But, although the amputation at these stages did not produce an observable effect upon

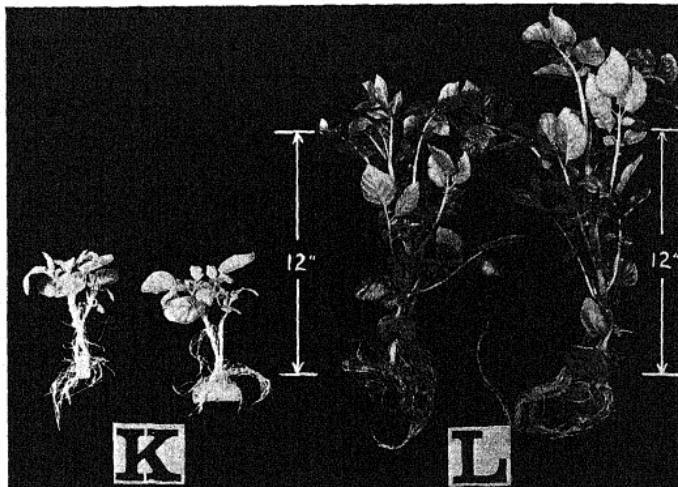


FIG. 4.—*K*, condition of amputated (left) and check (right) plants at time of amputation (May 31) when mother tubers were removed at stage 2; *L*, representative samples of same series on June 19; later in season the vine growth of amputated and check plants approximately equal.

the growth of vines, it will be seen from the data in table IB that it is possible that the yield of tubers was influenced, either unfavorably as in the case of Irish Cobbler, or favorably as in the case of Bliss Triumph.

YIELDS OBTAINED.—Although a yield record was obtained in the 1926 experiments, these data are not included in this report because of the failure to obtain adequate checks, as described in a previous paragraph. The plants in the 1927 experiments suffered from attacks of *aphis*, and the growth of many of the plants from midseason until the vines died was not normal, many plants dying and other plants

being obviously injured by the numerous spray applications that were needed. This resulted in many missing hills in the field; and, since in these experiments the arrangement of the plants in the field was a necessary feature in permitting dependable comparisons as to the final yield of amputated and check plants, the yield data for 1927 were excluded. In the 1928 experiment (which is to be considered the main one in this series), however, the conditions were favorable in all respects for normal growth; and, since the amputated lots had comparable checks in each case, the data are considered reliable and are presented in tables IA and IB.

In table IA the first four columns show the yield in grams obtained from plants from which the mother tubers were removed at stage 1, that is, when the sprouts were first emerging from the soil. Columns 1 and 2 show the results with Irish Cobbler, and columns 3 and 4 show those with Bliss Triumph. Each figure represents the yield in grams from a single plant. The average yields when mother tubers were not removed were 255 gm. for Irish Cobbler and 350 gm. for Bliss Triumph, while the yields per plant when mother tubers were removed were 86 gm. for Irish Cobbler and 113 gm. for Bliss Triumph. Table IA, columns 1, 2, 3, and 4 show that removing the mother tubers at the time of emergence of the sprout markedly reduced the yield. Furthermore, it will be noted (columns 2 and 4) that many of the plants from which mother tubers were removed at this early stage failed to develop at all. At this period of development the presence of the mother tuber is critical.

The yield data for stage 2 are shown in table IA, columns 5, 6, 7, and 8. Here again each figure represents the yield from a single plant. Columns 5 and 6 show that the average yield for Irish Cobbler was 328 ± 12.2 when the mother tuber was left on, and 274 ± 9.3 when it was removed. The difference (54 gm. per plant) is 3.5 times its probable error, and is therefore significant, showing that the removal of the mother tuber at this stage reduced the yield. In the case of the Bliss Triumph (columns 7 and 8) the corresponding values are 244 ± 11.4 for plants with mother tubers left on, and 197 ± 11.8 for plants with them removed. The difference (47 gm. per plant) is only 2.86 times its probable error, and is insufficient to show that amputation reduced the yield. Further experiments with this varie-

ty at this stage of development are necessary. It is apparent that the planting plan must be such as to place the amputated plants and their corresponding checks side by side in the field, in order to permit

TABLE IA
EFFECT OF AMPUTATION OF MOTHER TUBERS UPON YIELD

| MOTHER TUBERS REMOVED AT TIME OF EMERGENCE OF SPROUT (STAGE 1, FIG. 2A) | | | | MOTHER TUBERS REMOVED AFTER GERMINATION WAS COMPLETE AND LEAVES UNFOLDED (STAGE 2, FIG. 2B) | | | |
|---|--------------------------|---------------------------------|--------------------------|---|--------------------------|---------------------------------|--------------------------|
| IRISH COBBLER | | BLISS TRIUMPH | | IRISH COBBLER | | BLISS TRIUMPH | |
| Not removed, yield in gm. | Removed, yield in gm. | Not removed, yield in gm. | Removed, yield in gm. | Not removed, yield in gm. | Removed, yield in gm. | Not removed, yield in gm. | Removed, yield in gm. |
| 115 | 40 | 403 | 50 | 440 | 358 | 370 | 258 |
| 255 | 46 | 108 | 118 | 527 | 202 | 357 | 04 |
| 350 | 40 | 102 | 140 | 385 | 282 | 285 | 100 |
| 343 | 75 | 418 | 42 | 447 | 290 | 260 | 100 |
| 470 | 35 | 474 | 74 | * | * | * | 120 |
| 292 | 27 | 403 | 80 | 257 | 300 | 115 | 330 |
| 222 | 25 | 275 | * | 468 | 270 | 190 | 155 |
| 215 | 26 | 177 | * | 268 | 276 | 207 | 243 |
| 290 | * | 354 | * | 170 | 200 | 170 | 257 |
| 143 | 253 | * | * | 240 | 248 | 107 | 225 |
| 430 | 105 | 307 | * | 277 | 294 | 270 | 44 |
| 235 | 208 | 405 | * | 294 | 295 | 252 | 29 |
| 230 | * | 454 | * | 257 | 360 | 320 | 62 |
| 85 | * | 193 | * | 380 | 199 | 208 | 85 |
| 129 | * | 330 | * | 393 | 320 | 240 | 190 |
| 288 | 120 | 318 | 230 | 306 | 440 | 440 | 185 |
| 287 | 75 | 303 | 42 | 520 | 440 | 207 | 285 |
| 355 | 97 | 615 | 72 | 340 | 330 | 170 | 285 |
| 273 | * | 192 | 185 | 370 | 415 | 472 | 300 |
| 75 | 160 | 329 | 140 | 520 | 249 | 180 | * |
| 203 | 44 | 474 | 172 | 340 | 362 | 390 | 192 |
| 318 | 145 | 490 | 126 | 209 | 290 | 235 | 375 |
| 270 | 150 | * | 98 | 228 | 192 | 250 | 170 |
| * | 128 | * | * | 105 | 290 | 172 | 115 |
| Average 255 | Average 86 | Average 350 | Average 113 | 256 | 190 | 290 | 390 |
| | | | | 302 | 100 | 70 | 250 |
| | | | | 308 | 222 | 190 | 202 |
| | | | | 153 | 270 | 185 | 70 |
| | | | | 162 | 142 | 308 | * |
| | | | | 274 | 147 | * | * |
| | | | | 340 | 247 | 135 | 77 |
| | | | | 330 | 173 | 290 | 310 |
| | | | | 347 | 244 | 123 | 270 |
| | | | | 456 | 241 | 215 | 135 |
| | | | | 390 | 257 | 254 | 304 |
| | | Average 328 | Average 274 | Average 244 | Average 197 | | |
| | | P.E. ± 12.2 | P.E. ± 9.3 | P.E. ± 11.4 | P.E. ± 11.8 | | |

* Plants failed to develop; missing hills not included in computing averages and probable errors.

more critical comparisons regarding the influence of amputation at this stage upon yield.

The yields for the series in which the mother tubers were removed at stage 3 (when plants were half-grown) and stage 4 (when plants had attained maximum height and were in bloom) are shown in

table IB. Since the two varieties behaved quite differently with respect to the effect of the removal of mother tubers at these two stages, they will be discussed separately. The results with Irish Cobbler

TABLE IB
EFFECT OF AMPUTATION OF MOTHER TUBERS UPON YIELD
(CONTINUATION OF TABLE IA)

| MOTHER TUBERS REMOVED WHEN PLANTS WERE 10 INCHES HIGH, AND YOUNG TUBERS FORMING (STAGE 3, FIG. 2C) | | | | MOTHER TUBERS REMOVED WHEN PLANTS WERE NEARLY MAXIMUM HEIGHT, AND IN BLOOM (STAGE 4, FIG. 2D) | | | |
|--|--------------------------|------------------------------|--------------------------|---|--------------------------|------------------------------|--------------------------|
| IRISH COBBLER | | BLISS TRIUMPH | | IRISH COBBLER | | BLISS TRIUMPH* | |
| Not removed, yield in gm. | Removed, yield in gm. | Not removed, yield in gm. | Removed, yield in gm. | Not removed, yield in gm. | Removed, yield in gm. | Not removed, yield in gm. | Removed, yield in gm. |
| 200 | 105 | 135 | 253 | 130 | 154 | 85 | 200 |
| 160 | 154 | 140 | 170 | 117 | 30 | 58 | 68 |
| 192 | 290 | 122 | 125 | 100 | 47 | 106 | 124 |
| 59 | 130 | 242 | 200 | 183 | 146 | 182 | 125 |
| 58 | 200 | 202 | 208 | 125 | 180 | 125 | 155 |
| 140 | 177 | 150 | 249 | 134 | 225 | 142 | 95 |
| 402 | 226 | 302 | 275 | 102 | 187 | 232 | 198 |
| 193 | 160 | 186 | 253 | 247 | 107 | 138 | 116 |
| 222 | 290 | 333 | 204 | 251 | 190 | 140 | 167 |
| 270 | 265 | 274 | 262 | 118 | 155 | 166 | 166 |
| 240 | 170 | 116 | 180 | 173 | 204 | 90 | 180 |
| 250 | 70 | 106 | 366 | 155 | 170 | 148 | 190 |
| 140 | 254 | 150 | 144 | 135 | 130 | 210 | 234 |
| 233 | 215 | 176 | 204 | 196 | 142 | 116 | 154 |
| 150 | 190 | 78 | 157 | 103 | 103 | 160 | 225 |
| 223 | 165 | 127 | 201 | 112 | 117 | | |
| 190 | 144 | 252 | 93 | 103 | | | |
| 206 | 172 | 156 | 115 | 56 | 52 | Average 140 | Average 162 |
| 220 | 198 | 135 | 89 | 136 | 111 | | |
| 308 | 70 | 60 | 106 | 200 | 216 | | |
| 268 | 142 | 256 | 240 | 204 | 172 | | |
| 290 | 120 | 146 | 153 | 183 | 118 | | |
| 126 | 203 | 152 | 180 | 189 | 216 | | |
| 235 | 190 | 40 | 250 | 177 | 207 | | |
| 224 | 150 | 198 | 150 | 311 | 255 | | |
| 190 | 135 | 103 | 120 | 177 | 280 | | |
| 100 | 102 | 69 | 53 | 126 | 230 | | |
| 120 | 70 | 142 | 233 | 126 | 208 | | |
| 174 | 90 | 173 | 50 | 178 | 197 | | |
| 120 | 90 | 131 | 121 | 218 | 210 | | |
| 190 | 108 | 117 | 99 | 157 | 154 | | |
| 192 | 122 | 133 | 107 | 202 | 172 | | |
| 148 | 130 | 181 | 212 | 202 | 218 | | |
| 125 | 146 | 90 | 156 | 138 | 147 | | |
| 190 | 220 | 125 | 159 | 230 | 223 | | |
| 153 | 190 | 128 | 206 | 187 | 253 | | |
| 73 | 202 | 44 | 176 | 253 | 253 | | |
| 240 | 127 | 140 | 220 | 186 | 186 | | |
| 130 | 207 | 101 | 163 | 162 | 202 | | |
| 185 | 75 | | | 102 | 202 | | |
| 170 | 170 | Average 153 | Average 181 | 133 | 112 | | |
| 204 | 206 | | | 88 | 206 | | |
| 243 | 148 | | | 228 | 212 | | |
| 155 | 190 | | | 105 | 135 | | |
| 170 | 277 | | | 179 | 62 | | |
| Average 189 | | Average 166 | | 45 | 78 | | |
| | | | | 102 | 109 | | |
| Average 160 | | Average 164 | | | | | |

* Because of rotting of mother tubers of Bliss Triumph between stages 3 and 4, only 30 plants with firm mother tubers were available for amputation at this stage.

bler for stage 3 are shown in columns 1 and 2, table IB, and show the response from 45 pairs of hills, one plant of each pair being the check from which the mother tuber was not removed, and the other being the plant from which the mother tuber was removed at stage 3 (plants half-grown, fig. 2C). The average yield per plant when mother tubers were left on was 189 gm., and that for plants from which mother tubers were removed was 166 gm.

It was anticipated that probably only small differences would be found in the yields from plants with mother tubers not removed, as compared with plants from which they were amputated at these later stages of growth. Consequently, in transplanting after removal of mother tubers in the two series at stages 3 and 4, the amputated and check plants were placed adjacent to each other in pairs, in order that the soil conditions would be more nearly alike, and in order that the yield data could be compared by the simultaneous difference method employed by ENGLEDOW and YULE (7) and by use of the formula given by FISHER (8). Calculations made by ENGLEDOW and YULE's method show odds of only 13 to 1 for significance of the observed difference in this case. When the FISHER formula is applied to the same data, the value of "t" found was 1.77, which, as shown by the table for "t" values (FISHER 8, p. 137), corresponds to odds of less than 20 to 1. FISHER's requirement for a probability of $P=0.05$ would necessitate a value of about 2.0 in this case; the value actually found (1.77), therefore, falling short.

Taking into account the probabilities shown by these two methods, it seems that, while there is some evidence that the removal of mother tubers decreased the yield, the assurance is not complete, and further experiments must be made to permit a definite statement as to the influence of removal at this stage of development.

The response of Irish Cobblers to the amputation of mother tubers at stage 4 (plants nearly full height and in bloom) is shown in table IB, columns 5 and 6. In this case the average yield with mother tubers left on was 160 gm. per plant, and with them removed was 164 gm. Removing the mother tubers at this late stage of development had no effect upon the final yield of tubers.

The results obtained with Bliss Triumph variety at stages 3 and 4 are shown in columns 3, 4, 7, and 8 in table IB. In examining these

data a surprising result is obtained, namely, the plants from which the mother tubers were amputated gave larger yields than those from which they were not removed.

Thus (columns 3 and 4, table IB) the yield from plants from which the mother tubers were removed at stage 3 (half-grown) was 181 gm. per plant, while the yield from plants from which they were not removed was 153 gm. There were thirty-nine pairs of plants available for this comparison. When the data are treated by the method of ENGLEDOCK and YULE (7), it is found that the odds are 36 to 1 that the observed difference is significant. When use is made of the formula of FISHER (8), the value of "t" resulting from the calculation is found to be 2.42. When this value is fitted into FISHER's table of "t" values it is found that his requirement of a probability at least equal to $P=0.05$ has been satisfied, and that odds greater than 19 to 1 in favor of the view that the amputated series has given greater yields than the checks have been obtained.

A probable cause of this result may be found in the fact that, in the period following stage 3, the Bliss Triumph mother tubers that remained in contact with the sprout began to rot badly. When the time arrived to amputate them at stage 4, most of the plants had to be discarded because, upon inverting the pots, it was found that the mother tubers were either partly or completely rotten. Therefore, only thirty plants with firm mother tubers were available for the comparison at stage 4. The results of the amputation at stage 4 are shown in table IB, columns 7 and 8. Here again a greater average yield was found for the plants from which mother tubers were removed, that is, 162 for the amputated and 140 for the corresponding checks. STUDENT'S (19) method shows odds of 19.5 to 1 that this difference is significant, but the calculation by FISHER'S method gives a "t" value for the observed data amounting to 1.84, which is somewhat below the "t" value (about 2.0) that would be required by him for a probability of $P=0.05$, that is, 19 to 1. Thus, while the data partially support the view that contact with the mother tuber at this stage of development has reduced the yield of tubers, the odds are not sufficient to give assurance. The evidence at stage 4, however, tends to substantiate the similar condition found at stage 3.

It will be noted in tables IA and IB, that the check plants from which mother tubers were not removed gave yields that differed considerably in the four series. Thus the checks for the amputation at stage 1 gave an average yield for Irish Cobbler of 255 gm. (table IA, column 1); the checks for stage 2 yielded 328 gm. per plant (table IA, column 5); while for stages 3 and 4 the yields of check plants were 189 and 160 gm. respectively (table IB, columns 1 and 5). Since these were check plants (mother tubers not removed), amputation could not have been a factor, yet the differences in yield were even greater than the differences due to amputation. It should be remembered that the check plants grew in pots for different lengths of time in the four series, and that after amputation the plants were of necessity transplanted into different parts of the field in the four series. Consequently the conditions for growth were considerably different, and a difference in yield could be expected. These differences between the checks in the different series, however, do not vitiate the differences between the amputated and check plants in each series, since the experiment was arranged to make conditions within each series the same for both amputated and check, except for presence and absence of mother tubers. The high variability in yield of individual potato plants, and the differences observed between checks in the four series, emphasize the importance of the precautions taken to arrange the experiment so that the amputated plants and their corresponding checks could be compared directly with each other, under conditions that make these disturbing factors inoperative.

CHEMICAL COMPOSITION OF AMPUTATED MOTHER TUBERS

Analyses of the tissue obtained in amputating mother tubers at intervals after planting were obtained for all three years. Although, as previously described, the yield data were not suitable for comparisons in the 1926 and 1927 experiments, because of inadequate checks or interference with the planting plan after amputation, the plants from which mother tubers were removed had made apparently a normal growth up to the time of amputation, and consequently the analytical results from the amputated tissue should be of value, and are included.

SAMPLING METHODS.—As soon as the potato tissue was removed from the sprout the pieces were wiped with a moist rag, and peeled to remove the old epidermis and the suberized layer that had formed at the cut surface. The tissue was then either minced in a food grinder or chopped in a bowl. In the preliminary experiments in 1926 and 1927 it was found that, as the season progressed, the mother tubers became very high in water content; hence when the tissue was passed through a food grinder liquid was pressed out, and it was difficult to get a sample of the tissue which represented uniform proportions of solid and liquid. In the 1928 experiments, therefore, the potato tissue was chopped into fine pieces in a wooden bowl, and better sampling obtained by this method. As soon as the tissue was ready for sampling, weighed portions were removed for the moisture determination. To obtain a sample for the subsequent analyses for all constituents except sugar, weighed amounts (150–300 gm. per sample) were dropped (small quantities at a time) into boiling alcohol. The amount of alcohol used was adjusted so that the final concentration, taking into account the water content of the tissue, would be about 70 per cent. The tissue was stored in this concentration, but when the extractions for the analyses were begun, water was added to make the alcoholic concentration 50 per cent by volume. Three extractions at boiling temperatures were made, the liquid being decanted after settling. The tissue was then dried and ground to a fine powder which was extracted twice in the same manner, the decanted portions being added to the liquid obtained by the first three extractions.

Fifty per cent alcohol (by volume) was selected as the solvent for two purposes: first, to separate the starch from non-starch substances, as recommended by BRYAN, GIVEN, and STRAUGHN (4); and second, to separate protein from non-protein nitrogen. Working with the alfalfa plant, OSBORNE, WAKEMAN, and LEAVENWORTH (13) found that alcohol at 53 per cent (by weight) precipitated protein completely from the juice, and that 20 per cent (by weight) caused the precipitation of most of the protein present. In the present experiments it was found that very little if any protein was extracted by alcohol at a concentration of 50 per cent (by volume). Tests by such protein precipitants as colloidal iron, trichloracetic acid, and

tannin produced small precipitates in which only traces of nitrogen were present. Lead acetate precipitated appreciable quantities of nitrogen, but the results of VICKERY and VINSON (22) indicate the possibility that these were substances other than protein.

The extractions were made up to a definite volume after cooling, and this liquid was centrifuged, decanted, and aliquots taken for analysis. This was called the soluble portion, solubility in this case referring to 50 per cent alcohol (by volume) as the solvent.

The small amount of precipitate obtained in centrifuging the extract was added to residue from the extraction, and this, which was called the insoluble portion, was dried on a water bath and ground to a fine powder.

Separate samples of the fresh tissue were taken for the sugar determinations, and this tissue was also dropped into boiling alcohol, exactly as just described for the main sample except that calcium carbonate was added to the alcohol.

METHODS OF ANALYSIS.—For reducing sugars the MUNSON and WALKER method (2) was used, and the precipitated cuprous oxide was titrated with potassium permanganate, the permanganate value being determined by comparison with a sample of dextrose obtained from the Bureau of Standards. Sucrose was determined by inverting in the cold with hydrochloric acid (2). Ammonia was estimated by the aeration method after adjusting the solution to slight alkalinity. The apparatus of VAN SLYKE and CULLEN (21) was used and the aeration period was 2.5 hours. For amide, the liquid after ammonia aeration was neutralized with a drop or two of acid, and, after the addition of 1 cc. of concentrated hydrochloric acid for each 10 cc. of liquid, was hydrolyzed for 1.5 hours; the liquid was then transferred to an evaporating dish and the hydrochloric acid removed by evaporation. The liquid was then made slightly alkaline and again aerated in the VAN SLYKE-CULLEN apparatus, and the ammonia formed during the hydrolysis was estimated. For "basic" nitrogen, the liquid remaining after the amide aeration was used; phosphotungstic and sulphuric acids were added, and the liquid was heated to boiling. It was then cooled, made up to volume in a flask, mixed thoroughly, and allowed to stand over night. The precipitate was separated by the use of the centrifuge; to it were added the particles of precipitate that remained in the volumetric flask, and the com-

bined precipitates were washed twice with dilute phosphotungstic acid solution. The final precipitate was analyzed for nitrogen by the KJELDAHL method (2). The liquid decanted from the phosphotungstic precipitate was used for both the non-basic and amino-acid nitrogen, the non-basic by the KJELDAHL method, and the amino-acid gasometrically by the VAN SLYKE procedure (20). For the estimation of starch the diastase method with subsequent acid hydrolysis was used (2), but in this case saliva was found preferable to the malt extract because of the low "blank" value with saliva.

RESULTS OF ANALYSES.—The percentage composition calculated on the basis of fresh weight of the tissue is shown in table II and fig. 5. It will be noted that a rapid fall occurred in the percentages of all constituents except moisture, sugars, and soluble solids. Thus while the starch percentage dropped from about 10-15 to about 0.3-0.6 per cent (table II, column 8), the water content increased from about 70-80 to about 96 per cent. The various forms of nitrogen (table II, columns 11-17) all show a continuous reduction in percentage composition throughout the period of analysis, until finally the mother tubers removed at stage 4 were almost completely depleted of all forms of nitrogen, the percentage remaining being only about one-fifth or one-tenth of that present in the mother tubers at the time of planting. The change in the percentage of reducing sugar shows an interesting behavior in that, although there is a continuous loss of dry substance, the percentage of reducing sugar actually increased (table II, column 9). The details of these changes vary somewhat with the variety and with different years. There is a tendency for the percentage to be highest in midseason, that is, when the plants are about 10 inches high, and to recede again at the later stages. Another interesting feature is to be noted with respect to the percentage of solids soluble in 50 per cent alcohol. This tends to remain more nearly constant than any other constituent for which data were obtained. Thus, up to about stage 3 (stage 2 in the case of Bliss Triumph in 1928) the percentage of soluble solids was about 80 per cent of the value at the start of the experiment. Probably the increase in sugar explains this maintenance of a high soluble solids value in spite of the continuous loss of dry weight during the same period. Later in the season the percentage of soluble solids

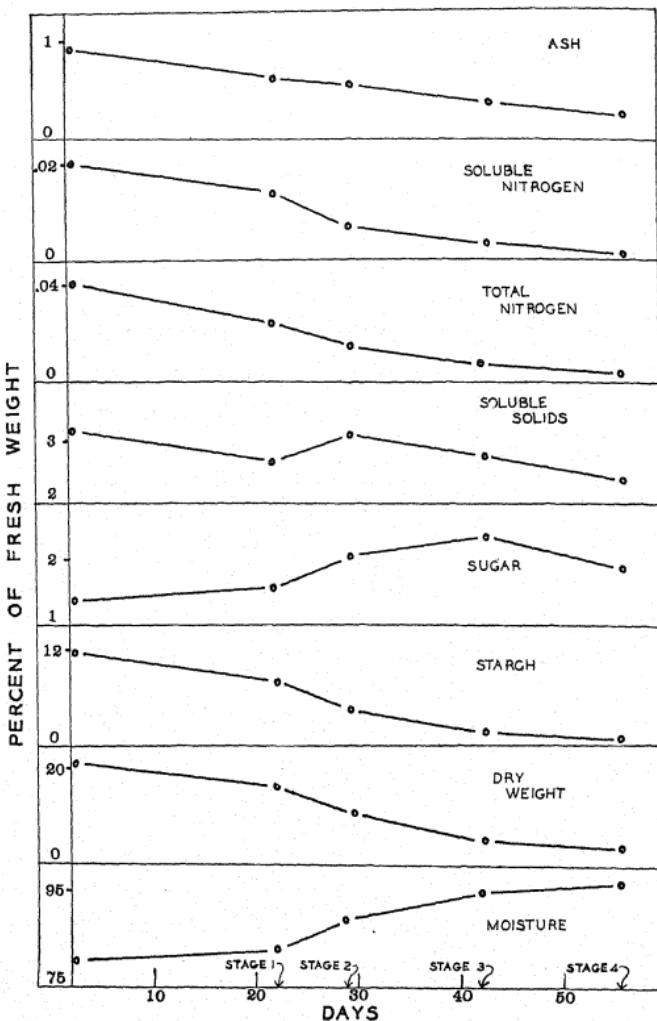


FIG. 5.—Composition of mother tuber tissue at beginning of experiment and at amputation at different stages of growth: points in circles over abscissa at 22 days show composition of mother tuber tissue removed at stage 1, those over the 29-day abscissa at stage 2, etc.; ordinates show percentages on fresh weight basis. Note change in percentage range for each constituent (left) to correspond to requirements of different constituents.

TABLE II
CHEMICAL COMPOSITION OF MOTHER TUBERS REMOVED FROM POTATO SPROUTS AT DIFFERENT INTERVALS AFTER PLANTING

| VARIETY AND DATE OF PLANTING | DATA REGARDING REMOVAL OF MOTHER TUBERS | | | | PERCENTAGE COMPOSITION ON FRESH WEIGHT BASIS | | | | | | | | |
|-------------------------------|---|------------------------------------|---|------------------|--|--------|-----------------|--------------------------|-------------------|---------|--------------|-------------|---------|
| | Date of removal | No. days after emergence of sprout | Condition of plant at time of removal | H ₂ O | Solids soluble in 50% alcohol | Starch | Reducing sugars | Cane sugar | | | Nitrogen (N) | | |
| | | | | | | | | Insoluble in 50% alcohol | NH ₃ N | Amide N | Basic N | Non-basic N | Amino N |
| Irish Cobbler, May 12, 1926 | Seed-pieces at start, before planting | 23 | No. days after emergence of sprout | 71.6 | 2.80 | 15.72 | 0.55 | 0.15 | 0.17 | 0.14 | 0.087 | 0.078 | |
| | June 4 | 2 | | 87.6 | 2.51 | 6.15 | 0.99 | 0.11 | 0.27 | 0.009 | 0.057 | 0.051 | |
| | June 14 | 13 | | 91.8 | 2.62 | 3.05 | 1.88 | 0.08 | 0.04 | 0.10 | 0.033 | 0.044 | |
| | June 21 | 19 | | 94.3 | 2.41 | 1.44 | 1.93 | 0.13 | 0.03 | 0.005 | 0.026 | 0.044 | |
| | June 29 | 27 | | 95.0 | 2.11 | 0.48 | 1.61 | 0.15 | 0.02 | 0.002 | 0.021 | 0.027 | |
| | July 7 | 35 | | 95.5 | 1.94 | 0.32 | 1.48 | 0.03 | 0.06 | 0.007 | 0.012 | 0.014 | |
| Irish Cobbler, April 22, 1927 | Seed-pieces at start, before planting | 36 | No. days after emergence of sprout | 80.4 | 2.81 | 10.61 | 0.50 | 0.16 | 0.21 | 0.023 | 0.076 | 0.066 | |
| | May 28 | 7 | Sprouts 2", high | 89.8 | 3.82 | 1.28 | 0.50 | 0.08 | 0.07 | 0.007 | 0.027 | 0.030 | |
| | June 16 | 26 | Sprouts 9", high | 95.3 | 2.04 | 0.35 | 0.24 | 0.04 | 0.03 | 0.002 | 0.010 | 0.015 | |
| | June 30 | 40 | Sprouts 13", high | 96.3 | 1.70 | 0.60 | 1.42 | 0.07 | 0.03 | 0.001 | 0.025 | 0.009 | |
| Irish Cobbler, May 2, 1928 | Seed-pieces at start, before planting | 22 | | 78.90 | 3.17 | 11.80 | 0.89 | 0.46 | 0.21 | 0.20 | 0.036 | 0.040 | |
| | | 0 | Emergence of sprouts (stage 1, fig. 2A) | 83.70 | 2.93 | 8.15 | 1.20 | 0.40 | 0.11 | 0.15 | 0.005 | 0.050 | |
| | May 31 | 7 | About 2" high, leaves expanded (stage 2, fig. 2B) | 88.60 | 3.15 | 4.89 | 1.42 | 0.72 | 0.07 | 0.08 | 0.005 | 0.030 | |
| | June 11 | 20 | About 10" above ground, half-grown (stage 3, fig. 2C) | 94.40 | 2.82 | 1.00 | 2.02 | 0.42 | 0.03 | 0.04 | 0.002 | 0.014 | |
| | June 25 | 56 | 34 Maximum height, in bloom (stage 4, fig. 2D) | 96.00 | 2.27 | 0.30 | 1.61 | 0.21 | 0.02 | 0.001 | 0.003 | 0.007 | |
| Bliss Triumph, May 2, 1928 | Seed-pieces at start, before planting | 22 | | 81.72 | 3.66 | 9.55 | 1.11 | 0.11 | 0.17 | 0.007 | 0.026 | 0.071 | |
| | May 24 | 7 | Emergence of sprouts (stage 1, fig. 2A) | 85.25 | 2.87 | 7.03 | 1.24 | 0.29 | 0.10 | 0.04 | 0.014 | 0.044 | |
| | May 31 | 29 | About 2" high, leaves expanded (stage 2, fig. 2B) | 91.90 | 3.35 | 3.33 | 1.86 | 0.46 | 0.06 | 0.05 | 0.002 | 0.018 | |
| | June 11 | 42 | About 10" above ground, half-grown (stage 3, fig. 2C) | 96.40 | 2.22 | 0.40 | 1.40 | 0.49 | 0.03 | 0.02 | 0.003 | 0.007 | |

dropped, but even at stage 4 its value was found to be 60-70 per cent of the original value.

The ash analyses, given in table III, are for the 1928 series only. Columns 2 and 5 show the percentage of ash obtained by ashing the portion of the tissue that was insoluble in 50 per cent alcohol; columns 3 and 6 show the ash percentages obtained by ashing aliquots of the liquid extract; and columns 4 and 7 represent the total ash obtained by adding the soluble and insoluble percentages. These analyses show a gradual fall in the different forms and in the total ash from the beginning to the end of the experiment. The ash,

TABLE III
ASH IN MOTHER TUBER TISSUE AMPUTATED FROM SPROUTS AT DIFFERENT
PERIODS OF GROWTH

| STAGE OF GROWTH AT TIME OF AMPUTATION | PERCENTAGE ON FRESH WEIGHT BASIS | | | | | |
|--|----------------------------------|----------|-------|---------------|----------|-------|
| | IRISH COBBLER | | | BLISS TRIUMPH | | |
| | Insoluble* | Soluble* | Total | Insoluble* | Soluble* | Total |
| Start of experiment..... | 0.425 | 0.455 | 0.880 | 0.285 | 0.316 | 0.601 |
| Stage 1 (fig. 2A)..... | 0.337 | 0.368 | 0.705 | 0.273 | 0.300 | 0.573 |
| Stage 2 (fig. 2B)..... | 0.302 | 0.310 | 0.612 | 0.195 | 0.197 | 0.392 |
| Stage 3 (fig. 2C)..... | 0.208 | 0.195 | 0.403 | 0.167 | 0.128 | 0.295 |
| Stage 4 (fig. 2D)..... | 0.020 | 0.160 | 0.180 | † | † | † |

* Percentage ash in the portion of tissue either soluble or not soluble in 50 per cent alcohol (by volume).

† Tissue not available for analysis (see text).

however, was not used up as completely as the dry weight or various forms of nitrogen, as shown in the previous paragraphs. Table III shows that at stage 3 approximately one-half of the ash was still present in the mother tissue. An analysis of the ash for various constituents was not made, but the ash has been retained for a subsequent analysis. The question as to the rate of utilization of the different ions is an interesting one, made especially so by the observations of MÜLLER (12) and of RAMSEY and ROBERTSON (14). In both contributions it is reported that potassium remains in the mother tuber and does not take part in the growth of the sprout.

CHANGES IN FRESH AND DRY WEIGHT OF INDIVIDUAL MOTHER TUBERS.—Since the seed-piece loses dry substance to the sprout, and also of course loses substance by its own respiration, and at the same

time gains in water content, it may be inquired whether, as a result of these two opposing factors, the absolute weight of the mother tuber will increase or decrease during the period of the experiment.

In the 1926 and 1927 experiments, no account was taken of the absolute change in weight of the mother tuber during the season. Hence, although the data could be calculated upon either the fresh or dry weight basis, it was impossible to determine the absolute amounts of materials leaving the individual mother tuber at any stage. In 1928 a special experiment was carried out to obtain data on this point (table IV). About 75 seed-pieces of each variety were carefully weighed on a small balance in order to obtain a weight of 28 gm. Ten of these were weighed at once, and the average weight was found to be 28.20 gm. for Irish Cobbler and 28.25 gm. for Bliss Triumph (table IV). The average deviation of the weight of any one seed-piece from the average weight of the ten was about one per cent. The other sixty-five pieces of each variety were then planted in the soil, and permitted to grow until the plants arrived at the various stages of development at which mother tubers were amputated in the main experiment. At each stage ten plants were lifted, the mother tubers removed, and after careful wiping with a moist cloth, the fresh and dry weight of each was obtained. The average values are shown in table IV. The changes in fresh weight of the individual mother tubers are shown in columns 2 and 3. It is seen that the absolute weight did not change much throughout the season, the Irish Cobblers losing in weight from 28.20 to 26.22 gm., and the Bliss Triumph mother tubers gaining in weight from 28.25 to 29.02 gm. The dry weight and water content per mother tuber are expressed in grams in columns 4, 5, 6, and 7 in table IV. Thus, while each Irish Cobbler seed-piece lost 5.24 gm. of dry matter (that is, from 6.43 to 1.19 gm.), it gained 3.26 gm. of water (that is, from 21.77 to 25.03 gm.). This observation, that the absolute weight of the mother tuber did not change to any great degree during the season, facilitated the interpretation of the data obtained from the samples of tissue. It showed that calculations upon the fresh weight basis are capable of showing the true changes in composition with respect to the various constituents. And, although the observations showing that this is true were not made until 1928, it seems likely

that the 1926 and 1927 data can be evaluated safely from the same point of view.

In the last six columns in table IV the changes in fresh weight, dry weight, and water content of the mother tubers at each stage of development have been calculated from these data, and expressed as a percentage of the amount in the mother tuber at the time of planting. Special attention is directed to the large percentage losses in dry substance. About 25-30 per cent was lost by the time the sprout emerged from the soil, about 50 per cent at the time germination was complete, and about 80 per cent at the time the plant was half-grown. It is not maintained, of course, that all the dry substance lost by the mother tuber was gained by the sprout. It is certain that part of it was lost by the respiration of the mother tuber tissue, and some of it might merely have leached out into the soil.

ABSOLUTE AMOUNTS OF VARIOUS CHEMICAL
CONSTITUENTS PER MOTHER TUBER

The data in table IV, showing the average fresh weight per mother tuber at each stage of development, combined with the data in table II, showing the percentage composition on the fresh weight basis, permitted a calculation of the absolute amounts of the various constituents in the individual mother tuber at each stage at which amputation was carried out. These data are shown for the 1928 experiments in table V.

By following down the columns in table V it is possible to observe the amounts of the various constituents present at the time of planting, and also the amount remaining in the mother tuber at each subsequent period of development.

One of the interesting features of table V relates to the utilization of starch between stages 2 and 3. At stage 2 leaves were fully formed, and the production of starch by the process of photosynthesis was going forward rapidly. It might have been expected that because of this there should have been less demand upon the stored starch, and yet during this period of high photosynthetic activity in the leaves the depletion of starch from the mother tuber was going forward at a rapid rate.

Another striking result was obtained with respect to the utiliza-

TABLE IV
FRESH WEIGHT, DRY SUBSTANCE, AND WATER CONTENT OF MOTHER TUBERS REMOVED FROM SPROUTS AT INTERVALS AFTER PLANTING

| DESCRIPTION OF MOTHER TUBERS REMOVED FROM SPROUT | PERCENTAGE OF AMOUNT IN MOTHER TUBER AT TIME OF PLANTING | | | | | | | |
|--|---|-------|---------|-------|---|-------|---------|-------|
| | FRESH WEIGHT PER MOTHER TUBER (GM.) | | | | WEIGHT OF H ₂ O PER MOTHER TUBER (GM.) | | | |
| | Cobbler | Bliss | Cobbler | Bliss | Cobbler | Bliss | Cobbler | Bliss |
| As planted | 28.20 | 28.25 | 6.43 | 5.47 | 21.77 | 22.78 | 100 | 100 |
| Emergence of sprout (stage 1, fig. 2A) | 27.40 | 28.88 | 4.45 | 4.17 | 22.96 | 24.71 | 102 | 105 |
| After germination complete and leaves expanded (stage 2, fig. 2B) | 27.46 | 29.24 | 3.20 | 2.79 | 24.26 | 26.45 | 97 | 96 |
| Plants 10 inches high and young tubers starting to form (stage 3, fig. 2C) | 26.30 | 29.02 | 1.31 | 1.08 | 24.99 | 27.93 | 93 | 90 |
| Plants in bloom and young tubers well developed (stage 4, fig. 2D) | 26.22 | * | 1.19 | * | 25.03 | * | 93 | * |

* Because of rotting of mother tubers of Bliss Triumph samples, a firm condition could not be obtained at this stage of development.

TABLE V
ABSOLUTE AMOUNTS OF SUBSTANCES PER MOTHER TUBER AFTER REMOVAL FROM SPROUTS AT INTERVALS AFTER PLANTING

| CONDITION OF MOTHER TUBERS REMOVED FROM SPROUT | WATER (gm.) | SOLIDS IN 50% ALCOHOL (gm.) | STARCH (gm.) | REDUCING SUGARS (gm.) | CANE SUGAR (gm.) | NITROGEN (N) | | | | | | | | | | | | | | | |
|--|----------------|--------------------------------------|-----------------|-----------------------------|------------------------|--------------------------------------|-------|------------------------------------|-------|----------------------------|-------|------------------|---------|------------------|---------|----------------------|---------|------------------|---------|---------|---------|
| | | | | | | Insoluble in 50% alcohol (gm.) | | Soluble in 50% alcohol (gm.) | | NH ₃ N (gm.) | | Amide N (gm.) | | Basic N (gm.) | | Non-basic N (gm.) | | Amino N (gm.) | | | |
| | | | | | | Cob- bler | Bliss | Cob- bler | Bliss | Cob- bler | Bliss | Cob- bler | Bliss | Cob- bler | Bliss | Cob- bler | Bliss | Bliss | | | |
| As planted. | 31.77122-779 | 0.804 | 1.034 | 3.358 | 2.698 | 0.231 | 0.314 | 0.130 | 0.178 | 0.059 | 0.041 | 0.048 | 0.00230 | 0.00590 | 0.00240 | 0.0190 | 0.0280 | 0.02010 | 0.02370 | 0.0153 | |
| Emergence of sprout (stage 1, fig. 2A) | 32.05824-717 | 0.803 | 0.835 | 2.233 | 2.290 | 0.359 | 0.358 | 0.110 | 0.084 | 0.030 | 0.041 | 0.030 | 0.0010 | 0.00120 | 0.00510 | 0.00110 | 0.01350 | 0.00860 | 0.01610 | 0.01270 | 0.0150 |
| After complete germination and leaves expanded (stage 2, fig. 2B) | 34.25626-450 | 0.805 | 0.981 | 1.343 | 0.974 | 0.390 | 0.544 | 0.198 | 0.135 | 0.019 | 0.017 | 0.022 | 0.0140 | 0.00060 | 0.00250 | 0.00200 | 0.00830 | 0.00380 | 0.00960 | 0.00520 | 0.00490 |
| Plants 10 inches high and young tubers starting to form (stage 3, fig. 2C) | 44.99027-935 | 0.744 | 0.644 | 0.287 | 0.116 | 0.531 | 0.466 | 0.110 | 0.116 | 0.008 | 0.011 | 0.0060 | 0.00050 | 0.00060 | 0.00160 | 0.00080 | 0.00370 | 0.00300 | 0.00370 | 0.00300 | 0.00200 |
| Plants in bloom and young tubers well developed (stage 4, fig. 2D) | 45.055-059 | * | 0.567 | | 0.079 | | 0.402 | | 0.055 | | 0.005 | | 0.0002 | | 0.0006 | | 0.0018 | | 0.0015 | | 0.0021 |

* At this stage of growth in the 1928 experiments, Bliss Triumph mother tubers were so badly rotted that a sufficient number could not be recovered.

tion of the sugars. Since the sugars are physiologically quite active, and therefore in condition for immediate use in respiration and growth, it might be expected that during periods of rapid growth these mobile substances would be used up rapidly and their concentration reduced to the minimum. Instead, however, the sugar actually increased during the period of rapid growth (see footnote 2). No doubt it was transported freely from the mother tuber, but this did not result in depletion, since apparently other compounds were broken down to sugar, and a high sugar level was maintained. The cane sugar situation is similar to that with reducing sugar, but the rate of loss of cane sugar was greater in the later stages of development (table V, columns 10 and 11).

The rate of disappearance of nitrogenous substances from the mother tubers is shown in table V, columns 12-25. The significant feature here is that all the forms of nitrogen for which analyses were made were utilized at about the same rate. It might reasonably have been expected that some forms would disappear faster than others, but the uniformity of the disappearance of these diverse groups is surprising. An estimate of this may be obtained by noting the number of cases in which the amount remaining at any stage is about one-half of that remaining at the previous stage. Out of forty-nine such pairs of values, thirty-one may be classed as essentially fulfilling this condition. Nothing of fundamental importance is claimed for this relation, which is quite accidental, since the stages chosen for amputation were merely empirical, but the coincidence serves to emphasize the facts observed. The data for 1926 and 1927 in table II also show a somewhat similar condition with respect to the rate of utilization of nitrogenous reserves.

BEHAVIOR OF DIFFERENT VARIETIES WITH RESPECT TO LOSS OF DRY WEIGHT

Because of the discovery of SELIBER (17) that the substance of the mother tuber of the variety Kruger is not utilized to any considerable extent by the sprout, a test was made with several varieties in order to see whether this behavior could be observed with American varieties. Tubers of ten varieties,⁴ in addition to the Irish Cob-

⁴ Appreciation is hereby expressed for the co-operation of Mr. WILLIAM STUART of the United States Department of Agriculture in supplying tubers of these varieties (except King Edward) for this experiment.

blers and Bliss Triumph used in the main experiment, were available for this test. Seed-pieces were prepared by first adjusting the fresh weights at approximately 20 gm. each, and then weighing them accurately on a balance. The average fresh weights are shown in table VI, column 2, and the corresponding dry weights in column 4. The seed-pieces were then planted and allowed to grow until the plants were about half-grown. The plants were then lifted, the mother tubers wiped carefully with a moist cloth, and the fresh and dry

TABLE VI
COMPARISON OF DIFFERENT VARIETIES OF POTATOES IN LOSS OF DRY WEIGHT
FROM MOTHER TUBERS

| VARIETY | FRESH WEIGHT OF MOTHER TUBER (GM.) | | DRY WEIGHT OF MOTHER TUBER (GM.) | | PERCENTAGE DRY WEIGHT LOST |
|-----------------------|------------------------------------|-------------------------------|----------------------------------|-------------------------------|----------------------------|
| | At planting | After amputation from sprout* | At planting | After amputation from sprout* | |
| Early Rose..... | 20.34 | 22.56 | 4.760 | 1.334 | 72 |
| King Edward..... | 20.33 | 24.07 | 3.923 | 0.956 | 76 |
| Green Mountain..... | 20.37 | 20.22 | 5.213 | 1.333 | 74 |
| Burbank..... | 20.19 | 19.37 | 4.656 | 1.080 | 77 |
| Russet Rural..... | 20.06 | 25.23 | 4.339 | 1.137 | 74 |
| Peerless Pearl..... | 20.66 | 21.69 | 4.741 | 0.696 | 85 |
| Beauty of Hebron..... | 19.60 | 22.74 | 4.543 | 1.286 | 72 |
| Early Ohio..... | 20.03 | 19.10 | 4.071 | 0.742 | 84 |
| Ehnola..... | 20.07 | 20.57 | 5.260 | 0.810 | 85 |
| McCormick..... | 20.85 | 21.49 | 3.621 | 0.859 | 76 |

* Amputation from sprouts after germination had been completed and plants were about 15 inches high.

weights of each again determined (table VI, columns 3 and 5). The right-hand column in table VI shows the percentage of the original dry weight lost by the mother tubers during the period of growth. It is apparent that none of the varieties showed a behavior similar to that of Kruger. All the varieties in the present test lost a large proportion of their dry weight. Early Rose and Beauty of Hebron lost 72 per cent of their original dry weight, and Peerless Pearl and Ehnola lost 85 per cent.

It is interesting to note also in this table the data available on the change in fresh weight of mother tubers during the season. In the previous discussion of the behavior of Irish Cobbler and Bliss Triumph, it was pointed out that only small changes in the weight

of the mother tuber took place, Irish Cobbler losing slightly in weight and Bliss Triumph gaining slightly. Table VI, columns 2 and 3, shows that of the varieties in this test, Green Mountain, Burbank, Peerless Pearl, Early Ohio, Ehnola, and McCormick showed such small changes that it may be doubted whether the amount of change is greater than the errors of measurement. Relatively large increases in fresh weight were shown by King Edward and Russet Rural. SELIBER (17) has emphasized the tendency of the tubers to gain in weight during the season, but in the present experiments the more usual tendency has been for the fresh weight to remain nearly constant. Probably the condition of the seed-piece at the time of planting and the weather conditions during growth are important factors.

Discussion

It should be emphasized that the results of this series of experiments were obtained with mother tubers 28 gm. in weight. This size was selected because from the literature it appeared that this is about the smallest size that can be depended upon to produce a plant with full vigor. And while in these experiments removal of mother tubers at midseason or later did not markedly reduce the final yield, it is possible that with smaller seed-pieces the influence of the mother tuber would have been exerted during the later periods of growth. Likewise, although a reduction in yield resulted from the removal of mother tubers after germination was complete and leaves were fully formed, it is possible that if large seed-pieces had been used, and if large supplies of stored materials had been available during the early stages of germination, the young plant might have become independent of the mother tuber at an earlier stage. Further work regarding the relation of size to the stage of development at which contact with the seed-piece is no longer critical for growth of the sprout would be desirable.

It may have been noted that the analytical data are presented on the fresh weight and the "per tuber" basis, but not on the dry weight basis. The results of the analyses in these experiments are peculiarly well fitted to emphasize the importance of the selection of the basis on which chemical analyses are calculated, and that erroneous conclusions may result from the use of a basis that is un-

suitable for the purpose. It is clear that in the present case the "per tuber" basis is the best one to show the rate of utilization of the stored substances, since in this way we deal with absolute amounts. The fresh weight basis is nearly as good, since in this particular case the fresh weight of a seed-piece is nearly constant throughout the period of sampling. If the information in table V (showing the absolute amounts of various constituents per mother tuber), and that in table IV (showing the dry weight per mother tuber) is used to calculate the percentage composition on the dry weight basis, some very misleading results are obtained. This method of calculation shows, for example, that at stage 2 the starch percentage of the Irish Cobblers was about 19 per cent lower than at the beginning, when as a matter of fact about 60 per cent of the starch had been lost by that time. Again, this method of calculation shows that little change in amino acid content had taken place between the beginning of the experiment and stage 3; but the mother tuber had lost, in fact, 83 per cent of this constituent. This confusion results from the condition that as the substances are used up the dry weight itself also falls, and the percentage change on this basis depends upon the relative rate of fall of the two changing values.

The observations (table IB, columns 1 and 2) that in midseason it appears possible that the Irish Cobbler plants had become completely independent of the mother tubers, and that amputation may not have influenced the yield unfavorably, are not in accord with the observations of SIKORSKI (18) and SELIBER (17). It must be remembered, however, that they suggest the importance of the mother tuber as an organ for water storage, and this factor would come into play only in periods of drought. In the present experiments there was an abundance of moisture in the soil at all times, and therefore no opportunity was afforded to test their hypothesis. The fact that in the preliminary experiments removal of the mother tuber by cutting across the base of the stem with a knife, or amputating by twisting, induced wilting of the plant even under moist conditions, lends support to the view that the water stored in the mother tuber might become an important factor.

The unfavorable effect upon the yield of Bliss Triumph which was obtained when the mother tubers were allowed to remain in con-

tact with the plant from stage 3 to stage 4 (table IB, columns 3 and 4) may have resulted from toxic materials formed during the rotting of the tubers. The reports of BREAZEALE (3) and COLLISON (5) emphasize the toxic effects of plant tissue during certain stages of decomposition. If toxic substances were formed during the rotting of mother tubers, the conditions for their absorption by the plant would be favorable because of the close connection at the base of the stem between the mother tuber tissue and the sap-conducting vessels.

DE VRIES (6) believed that the mother tuber furnished nutrients to the sprout until germination was complete, after which time its substance was transferred exclusively to the newly developing tubers. LUDWIG (11) adopts this as an argument against large tubers for seed-pieces, on the basis that it is not an economical method, since much of the starch in the large tubers merely passes out of the old into the young tubers, and at the end of six months is harvested a second time. There is evidence against these views in the results of the present experiments. Fig. 2 shows that at stage 2 very few young tubers had begun to form, and that even at stage 3 the new tubers were still small. The analyses (tables II and V, and fig. 5) show that at stage 3 nearly all of the starch and various forms of nitrogen had already been exhausted from the mother tuber; in fact, only 20-25 per cent of the original dry weight still remained, and the subsequent analyses showed that not much more transference from the mother tuber was to take place. Most of the materials destined to leave the mother tuber did so within about forty days from planting, or about twenty days from the date of emergence of sprout. We are not justified in inferring from this that the small amount of substance left at this stage does not exert a considerable influence upon the later growth of the plant, but it suggests that this influence is qualitative, and not due to the quantity of starch or other materials furnished.

This brings up the question as to the nature of the chemicals that pass from the mother tuber to the sprout and strongly influence its growth. Are these materials merely food stuffs, or are they special growth-promoting substances, such as have been postulated by APPLEMAN (1)? The present experiments seem competent to show that materials ordinarily regarded as food stuffs, such as sugar,

amides, amino-acids, etc., are used up rapidly and disappear from the mother tuber, and we must assume that they constitute an important part of the contribution made by the seed-piece. But the experiments are not capable of showing that special substances do not pass also, and that these do not exert an important function in regulating the use of the food stuffs that are translocated. We may regard the situation as analogous to that found by REED (15) in the twigs of lemons and other plants, in which two factors are considered to be operating jointly: first, stored food which (when in the proper condition) can support growth; second, special substances which (in small quantities) influence the utilization of these food materials in growth. REED speaks of them as substances which catalyze the growth process. In any event it is clear that the methods of analysis used in my experiments are not capable of showing the presence of special growth-promoting substances. The use of culture solutions would offer a more favorable method of experimentation; and the results of LUDWIG (11), showing the influence of salt content of the culture solution in modifying the rate of utilization of the stored foods in the tuber, are suggestive of the capacity of small amounts of one substance to control the utilization of a relatively large quantity of other substances in growth.

Summary

1. A series of experiments, in 1926, 1927, and 1928 is reported, in which the mother tubers were amputated from potato plants at intervals after planting, in order to observe the effect of this removal upon the subsequent growth and yield of the plant, and to note at what stage of development the young plants became independent of the food reserves of the seed-piece.

2. The seed-pieces were planted in the bottoms of pots which were then buried in the soil; when the sprouts had reached certain sizes the pots were removed and inverted, exposing the mother tuber, which was then amputated; the pot was discarded and the well-rooted plant replaced in the soil. The checks consisted of an equal number of plants which were removed from the pots at the same time but which were allowed to retain the mother tubers.

3. The mother tubers were removed at the following periods of

development: stage 1, when the sprouts first emerged from the soil and before leaves had expanded; stage 2, when plants were about 2 inches and had leaves fully expanded; stage 3, when plants were about 10 inches high and young tubers were forming on well-developed stolons; stage 4, when plants had attained approximately maximum height and were in bloom.

4. The effect of amputation upon subsequent growth is shown by photographs, and by tables of the yield. Plants from which the mother tubers were removed at stage 1 gave a yield of tubers only about one-third of that of the checks; removal of the mother tuber at this stage was critical and many plants were not able to survive. The plants from which mother tubers were removed at stage 2 gave a yield which was about 80 per cent of that of the corresponding checks, but the difference in yield was significant statistically, indicating that at this stage of development the young plant has not yet become independent of the stored food in the seed-piece. With respect to the effect of amputation at stage 3, the two varieties used (Irish Cobbler and Bliss Triumph) gave different responses. In the case of Irish Cobbler, plants from which mother tubers were removed showed a reduction in yield per plant, but the odds that this difference was significant were not high, leaving the issue in doubt. In the case of Bliss Triumph, the amputated plants gave a higher yield than the checks, indicating that contact with the mother tuber had been detrimental. It is suggested that this result was due to the rotting of the mother tubers between stages 3 and 4, with the production of toxic substances which were absorbed from the seed-piece at the base of the main stem and unfavorably influenced the subsequent growth. This effect was not observed in the case of Irish Cobbler seed-pieces, since they remained firm between stages 3 and 4. Removal of mother tubers at stage 4 produced no effect upon the yield of Irish Cobbler, but again the yield of Bliss Triumph was slightly greater with mother tubers off than with them on. The difference in this case, however, was not large enough to be conclusive statistically.

5. It appeared possible that the sprout became independent of the mother tuber at stage 3 (about 10 inches high). Most of the storage materials that were destined to leave the mother tuber had

already been used up. Further experiments are required to show conclusively whether contact with the mother tuber at this or later stages is beneficial; and if so whether the advantage results from the transfer of the small amount of food materials still available at that time, or from the water storage relation existing between seed-piece and sprout.

6. These experiments were carried out with mother tubers weighing 28 gm. each. It is possible that with smaller seed-pieces the sprout would have remained dependent on the mother tuber for a longer period, and that with larger pieces amputation could have been carried out at an earlier period without interfering with subsequent development.

7. The amputated mother tissue was subjected to chemical analyses in order to determine the rate at which stored substances left it, and to note any correlation which might exist between the composition at any stage of development and the subsequent behavior. A rapid loss of substance from the mother tuber was noted. By the time the sprout emerged, about one-fourth to one-third of the dry weight was lost; about one-half remained at stage 2; and at stage 3 the mother tubers had been depleted of nearly 80 per cent of the original dry weight. The extent of the depletion varied with different lots, in different years, and with different varieties, but ranged from 70 to 85 per cent. Starch and the various forms of nitrogen (both soluble and insoluble) were steadily used up; but the sugar concentration was maintained at a high level, so that, even though the dry weight had been reduced to a low level the sugar percentage on the fresh weight basis was greater at all subsequent stages than it had been at the time of planting.

8. The analyses showed that different groups of nitrogenous substances, such as insoluble, soluble, ammonia, amide, amino, basic, etc., were removed from the mother tuber at approximately the same rate. There was no evidence of one form being more readily available for growth than other forms.

9. Although between stages 2 and 3 the foliage was active photosynthetically, large demands upon the organic substance of the mother tuber were made during this period. A "sparing" action

upon stored foods in the underground part because of food manufacture in the tops of the plant was not noted.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

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GERMINATION AND KEEPING QUALITY OF PARSNIP SEEDS UNDER VARIOUS CONDITIONS¹

HILDA C. JOSEPH

(WITH TWO FIGURES)

Introduction

In several ways parsnip seeds furnish especially favorable material for the study of the effect of storage conditions upon longevity. They are short-lived, and when stored in bags in a laboratory the vitality drops about 20 per cent during two years and 60 per cent during three years of storage; hence the differential effect of storage conditions is quite evident within two years. They withstand extreme drying without injury to their vitality, which makes it possible to study the effect of a wide range of water content. They germinate with reasonable speed over a considerable range of temperature.

The experiments reported in this paper have been conducted for the purpose of finding a method by which parsnip seeds could be stored for several years without losing their vitality. Such a method would be of advantage to seedsmen in enabling them to avoid an annual discard of seeds left over from the previous year. It is also hoped that a method worked out for storage of parsnips might be applicable with modifications to other short-lived seeds of commercial importance, such as cabbage, onion, and a number of the coniferous seeds.

The problems which presented themselves in connection with this study of parsnip seeds were mainly the following: (1) Is the seed material as harvested and sold by the seed grower uniform enough for experimentation, or do the ripe and green seeds of each shipment require separate investigation? (2) What are the optimum temperature requirements for the germination of parsnip seeds, and does the optimum germination temperature remain unchanged when the seeds become older? (3) What influence have moisture content, stor-

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age temperature, humidity of storage chamber, fluctuation of humidity, and aeration upon the keeping quality of the seeds? (4) In which way or ways do the factors mentioned under (3) influence the viability of parsnip seeds?

Discussion of literature

A number of investigations have been made to determine the influence of various storage conditions upon the keeping quality of seeds. This discussion will be limited, however, to the reports of DUVEL (1), HEINRICH (2), MAQUENNE (3), NAKAJIMA (4), and TILLOTSON (5), which bear directly on the work reported in this paper.

The paper of MAQUENNE is the only one in which experiments with parsnip seeds are reported. MAQUENNE stored parsnips in vacuum for two years at room temperature. During this period the seeds retained their viability completely, while a control lot stored in a paper bag lost all vitality during the same period. Excessive drying, according to MAQUENNE, is the only way of successfully retaining viability in certain seeds, because only when the last traces of water are liberated from the seed under the action of the vacuum is a state of "supermaturation" reached. He defines supermaturation as "a new state of equilibrium established between the enzymes and the substances they condense," and speaks of it as a state of suspended life.

Since MAQUENNE's experiments ran only a little more than two years (1899-1902), it remains to be proved whether he really was able to produce a state of suspended life in his experimental material, or whether the life processes were only retarded enough to leave the final percentage of germination unchanged for a certain time. He does not give data to show whether the germination energy as well as the germination power remained constant. He also fails to show that there is no other way of storing by which vitality could be maintained in short-lived seeds. Since the data given are based on quantities of 2-4 gm. of seeds which were originally not of a very high quality (51 per cent of vital seeds at the beginning of the experiment), it seemed desirable to investigate the keeping quality of a larger number of parsnip seeds of good quality under a variety of storage conditions.

DUVEL (1) worked with a number of vegetable seeds, which he

stored under various climatic conditions in different places of the United States, such as Mobile, Alabama; Wagoner, Indian Territory; and Ann Arbor, Michigan. He determined the effect of various degrees of atmospheric humidity and temperature on the keeping quality of his material, and worked out different methods of packing and sealing seeds as a protection against the influence of high atmospheric humidities. One of his main conclusions is that "as a factor detrimental to vitality, moisture is of far greater importance than temperature."

DUVEL was working with atmospheres of high constant humidity and widely fluctuating humidities without distinguishing between the effects of the two. Later TOUMEY (6) emphasized the detrimental effect of variations in humidity, even when the moisture content reached at different times was not very high, making the following statement:

Even the most resistant species suffer when kept over summer in a loft where the moisture content of the seed is likely to vary with variations in the humidity of the air.

This quotation applies to forest seeds, especially to those of the coniferous type, and is taken from the chapter on seed storage, which contains very good general information as to optimum storage conditions for various tree seeds.

HEINRICH (2) has made significant contributions to our knowledge of the effects of storage conditions upon the vitality of seeds of cereal and forage crops. He determined the rate at which these seeds absorb water from a saturated atmosphere, also the total amount thus absorbed. He also found the "critical moisture content" for seed storage. With moisture content above the critical point there is rapid degeneration of the seeds in storage, while with moisture content below it the vitality is little modified by a considerable period of storage. Discovery of the critical moisture content is one of the very important contributions to our knowledge of seed storage. It is also a fact that has been largely overlooked by later investigators.

TILLOTSON (5) shows the favorable influence of dry air-tight storage upon the retention of vitality by coniferous seeds. His method avoids the injurious effects of fluctuating moisture content. He did not determine the exact moisture content of the seeds, but the

excellent keeping quality shows that they were below HEINRICH'S critical moisture content.

NAKAJIMA's experiments (4), published in 1927, are interesting because of the methods used for drying the seeds. In addition to drying over desiccating agents, he mixed quicklime or calcium chloride with the seeds, and varied the amount of the drying agent added to suit the amount of desiccation endured by the various seeds, thus avoiding excessive or injurious drying. He considers his method of controlled drying in closed chambers superior to "air-dry" air-tight storage.

Methods and material

In these experiments one variety of parsnip, commercially called Hollow Crown, was used exclusively. The supply was obtained from two different commercial seed firms in three shipments in the fall of 1924, and from one firm in one shipment in the fall of 1925. The shipments of 1924 (no. 24266, no. 24702, and Hollow Crown Guernsey) were lacking in uniformity, and were therefore separated into ripe and green lots. The shipment of 1925 appeared to be uniform enough to remain unselected. The total number of seeds handled in all experiments approximated 85,000.

All germination tests were carried out in electrically heated ovens, the temperature of which was automatically regulated. The seeds were placed on three thicknesses of filter paper in Petri dishes, in lots of 100 seeds in each dish. The seeds were left unsterilized after it had been determined that the loss, resulting from treatment with various concentrations of uspulun, was more severe than that caused by molding of the seeds. Alternations of temperature were effected by transferring the seeds from one oven to another, with the longer period (5:00 P.M.-9:00 A.M.) at the lower temperature.

The seeds were stored in glass bottles with rubber stoppers. When seeds with reduced moisture content were stored, the stoppers were cemented in the bottles and coated with DeKotinsky cement. Either the germination chambers just mentioned or an ice chest served as storage chamber. In each chamber the seeds remained in darkness except for the short intervals when the doors were opened. The moisture determinations were made on unmacerated seeds in ovens at 103° C., and in a vacuum oven at 72° C.

Experimental results

Before storage experiments were begun, preliminary studies were made with the different samples of Hollow Crown seeds: (1) to determine the vitality and optimum germination temperature of fresh seeds; (2) to determine the moisture content of air-dry green and ripe seeds; (3) to improve the germination of green seeds by artificial drying.

The vitality of ripe seeds (brown seed coats) proved to be superior to that of seeds not fully ripe (green seed coats). There is no specific optimum germination temperature for ripe or green seeds of recent harvest. As table I shows, they germinate equally well at

TABLE I
GERMINATION OF FRESHLY HARVESTED PARSNIP SEEDS; COMPLETE
GERMINATION REQUIRED 21 DAYS IN EACH CASE

| COLLECTION (1924) | COND- ITION OF SEED | NO. OF SEEDS IN EACH TEST | PERCENTAGE GERMINATION AT | | | | | |
|--------------------------|---------------------------|------------------------------------|---------------------------|--------|--------|--------|----------------|---------------|
| | | | 15° C. | 20° C. | 27° C. | 32° C. | 15°- 20° C. | 20- 32° C. |
| Hollow Crown no. 24702. | Ripe | 2×100 | 94 | 88 | 83 | Molded | 87 | 82 |
| Hollow Crown no. 23266. | Ripe | 2×100 | | 95 | 94 | Molded | | 94 |
| Hollow Crown unselected. | Ripe | 4×100 | | 95 | | | | |
| Hollow Crown no. 24702. | Green | 2×100 | 70 | 35 | 65 | Molded | | 73 |
| Hollow Crown no. 24266. | Green | 2×100 | 72 | 77 | 68 | Molded | 70 | 79 |

constant or alternating temperatures between 15° and 27° C., while 32° C. is too high for both kinds of seeds.

Simultaneously with the germination tests, moisture determinations were conducted, the results of which are given in table II. They show that in each of the samples tested the green seeds contain a greater amount of hygroscopic moisture than the ripe seeds.

In an attempt to improve the germination of green seeds by lowering their water content to that of ripe seeds, different lots of unripe seeds were dried in various ways (table III). From the data in table III one may draw the following conclusions: (1) Germination of green seeds can be favorably affected by drying in a vacuum oven at 60° C. for four days. This result was confirmed by similar tests with six more series of samples. The average improvement of germination of dried over undried seeds was 16.6 per cent. (2) There is no

direct relationship between water content and germination quality, since all green seeds with improved germination have a very much lower water content than ripe seeds that germinate equally well. (3)

TABLE II

MOISTURE CONTENT OF FRESHLY HARVESTED, AIR-DRY PARSNIP SEEDS;
NUMBERS REPRESENT AVERAGE OF THREE TESTS

| METHOD OF DRYING | NO. OF SAMPLE | AMOUNT OF HYGROSCOPIC MOISTURE IN PERCENTAGE OF DRY WEIGHT | |
|---------------------------|---------------|--|-------------|
| | | Ripe seeds | Green seeds |
| Vacuum 72° C. | 24702 | 6.33 | 7.46 |
| Vacuum 72° C. | 24266 | 6.13 | 6.50 |
| Access of air 94° C. | Guernsey | 6.83 | 6.90 |

The method of drying is of importance. Drying at room temperature or temperatures between 72° and 85° C. does not affect the germination quality either favorably or unfavorably, while at temperatures above these a marked injury is noticeable.

TABLE III

GERMINATION OF GREEN PARSNIP SEEDS DRIED IN VARIOUS WAYS; NUMBERS REPRESENT AVERAGES OF FIVE TESTS WITH 100 SEEDS EACH

| DRY TEMPERATURE | ORIGINAL MOISTURE CONTENT | LOSS OF MOISTURE | FINAL MOISTURE CONTENT | PERCENTAGE GERMINATION AT 20° C. |
|--|---------------------------|------------------|------------------------|----------------------------------|
| Undried seeds, fresh. | 7.4 | | 7.4 | 72.0 |
| Seeds kept at room temperature for 4 months in paper bag. | 7.4 | 2.8 | 4.6 | 64.2 |
| Fresh seeds dried at 60° C. in vacuum oven for 4 days. | 7.5 | 5.6 | 1.9 | 83.0 |
| Fresh seeds dried at 72° C. in vacuum oven for 4 days. | 6.8 | 7.0 | 1.8 | 74.0 |
| Fresh seeds dried at 90° C. with access of air 4 hours. | 7.4 | 6.1 | 1.3 | 78.0 |
| Fresh seeds dried at 95° C. with access of air 4 hours. | 6.5 | 5.6 | 0.9 | 23.0 |
| Fresh seeds dried at 103° C. with access of air 2 hours. | 6.8 | 4.2 | 2.6 | 51.0 |

This heat injury may be temporary only, provided the seed material is air-dry at the start and well ventilated during the drying. Table IV shows a slight temporary heat injury obtained in five sam-

ples of ripe seeds dried at 87° C. The seeds had completely recovered after one month. On the other hand, a complete destruction of viability occurs even at the optimum drying temperature of 60° C. when the samples are kept in sealed containers while they are heated.

After the percentage of vital seeds in freshly harvested parsnips and the optimum temperature or temperature range for germination had been found, storage experiments were set up in order to determine the influence of humidity, temperature, and ventilation upon the viability of stored seeds, and to find an improved method of storage. The seeds were arranged in the following series: (1) Air-dry seeds of known moisture content were stored in paper bags at

TABLE IV

EFFECT OF HIGH TEMPERATURES ON PARSNIP SEEDS KEPT IN OPEN AND IN CLOSED CONTAINERS; NUMBERS REPRESENT AVERAGE OF FIVE TESTS WITH 100 SEEDS EACH

| MATERIAL | DRYING TEMPERATURE | CONDITION OF SAMPLE | GERMINATION AT 20° C. | |
|--------------------------------|--------------------------------------|---------------------------|--------------------------------|-----------------|
| | | | Immediately | One month later |
| Hollow Crown Guernsey, ripe | 87° C. $4\frac{1}{2}$ hours | Weighing bottle uncovered | 84.0 | 96.0 |
| | 87° C. $4\frac{1}{2}$ hours | Weighing bottle sealed | None | None |
| | 60° C. $4\frac{1}{2}$ hours | Weighing bottle uncovered | 96.7 | 95.8 |
| | 60° C. $4\frac{1}{2}$ hours | Weighing bottle sealed | None | None |

room temperature and in an ice chest (5° – 7° C.); these seeds were to serve as controls for the following samples. (2) Air-dry seeds of known moisture content were stored in tightly stoppered bottles at 25° and at 5° C. (3) Seeds, the moisture content of which had been reduced to different degrees at various high temperatures, either with access of air or in vacuo, were stored in tightly stoppered bottles at 25° and 5° C. (4) Seeds were treated similarly to those mentioned under (2) and (3), but stored at temperatures between and below those recorded there. This last series is still in progress and will be discussed in a later paper.

When samples of seeds were taken out of the different storage conditions three years later, it was found that the temperature requirements for germination had changed. Green seeds from storage at ice box temperature showed a definite optimum germination at

15° C., instead of growing equally well at constant and alternating temperatures between 15° and 27° C. (table I). Samples of no. 24266, for instance, which had been stored in a paper bag in the ice chest, showed an average germination of 84 per cent at 15° C., 74 per cent at 20° C., and only 58 per cent at 15°-25° C. Green seeds stored at room temperature were all dead, so that all tests remained without results.

Ripe seeds had retained part of their vitality in all storage conditions. Table V shows how the optimum germination temperature had changed in various lots. All samples which had retained a high

TABLE V
CHANGE IN TEMPERATURE REQUIREMENTS FOR GERMINATION OF RIPE
PARSNIP SEEDS DURING STORAGE

| MATERIAL | WATER CONTENT | METHOD OF STORAGE | OPTIMUM GERMINATION TEMPERATURE (°C.) |
|-----------------------|---|------------------------------------|---------------------------------------|
| Ripe, fresh | 6.33 and 6.13 | | 15 or 20 |
| Ripe, 3 years old. | Fluctuating 6.33-5.60 | Room temperature, paper bag | 15-25 alt. |
| Ripe, 3 years old. | Constant 6.13 and 6.83 | Room temperature, sealed bottle | 15-25 alt. |
| Ripe, 3 years old. | Reduced to various degrees, constant | Room temperature, sealed bottle | 15 |
| Ripe, 3 years old. | Fluctuating 6.33-9.10 | Ice chest, paper bag | 15 |
| Ripe, 3 years old. | Constant 6.13 and 6.83 | 5° C., sealed bottle | 15 |
| Ripe, 3 years old. | Reduced to various degrees, constant | 5° C., sealed bottle | 15 |

percentage of vitality (as may be seen from tables VI and VII) germinated best at 15° C., while seeds of lower vitality (tables VI and VII) needed alternation of temperature.

Tables VI-VIII show the decrease in germination of the various samples under different storage conditions during the first three years of storage.

The effect of storing seeds in paper bags where they are exposed to variations in atmospheric humidity and temperature is shown in table VI. Ripe seeds retained their viability much better when stored in an ice chest as compared with laboratory storage, in spite of the high humidity in the ice box, which increased the moisture content of the seed considerably. Green seeds lost their vitality faster than ripe seeds under the same cool and humid conditions.

A storage of air-dry seeds in sealed containers under similar

temperature conditions proved very unfavorable at high as well as at cool temperatures (table VII), which shows that fluctuations in

TABLE VI

LOSS OF VITALITY IN VARIOUS COLLECTIONS OF PARSNIP SEEDS DURING STORAGE
AT ROOM TEMPERATURE AND IN ICE CHEST IN PAPER BAGS

| COLLECTION | NO. OF SEEDS USED | MOISTURE CONTENT | LENGTH OF STORAGE PERIOD | STORAGE TEMPERATURE | PERCENTAGE GERMINATION | AT OPTIMUM GERMINATION TEMPERATURE (°C.) | PERCENTAGE LOSS OF VITALITY |
|--|-------------------|------------------|--------------------------|---------------------|------------------------|--|-----------------------------|
| Hollow Crown ripe, no. 24202, 1924..... | 2×100 | 6.33 | Fresh | | 94.0 | 20 | None |
| | 3×100 | 5.60 | 2 years | Room temperature | 72.3 | 20 | 20.6 |
| | 4×100 | 5.60 | 3 years | Room temperature | 58.0 | 15-25 | 39.3 |
| Hollow Crown ripe, no. 25266, 1924..... | 2×100 | 6.13 | Fresh | | 95.0 | 20 | None |
| | 3×100 | 9.10 | 2 years | Ice chest | 87.0 | 20 | 8.5 |
| | 4×100 | 9.10 | 3 years | Ice chest | 84.0 | 15 | 11.6 |
| Hollow Crown green, no. 24266, 1924..... | 2×100 | 6.5 | Fresh | | 77.0 | 20 | None |
| | 3×100 | 8.8 | 2 years | Ice chest | 54.0 | 20 | 29.9 |
| | 4×100 | 8.8 | 3 years | Ice chest | 46.0 | 15 | 40.4 |
| Hollow Crown unselected, 1925..... | 2×100 | 6.8 | Fresh | | 94.0 | 20 | None |
| | 3×100 | 8.6 | 1 year | Ice chest | 90.3 | 20 | 4.0 |
| | 4×100 | 8.6 | 2 years | Ice chest | 78.0 | 15 | 17.1 |

TABLE VII

KEEPING QUALITY OF PARSNIP SEEDS WITH ORIGINAL WATER CONTENT STORED
IN TIGHTLY STOPPED BOTTLES AT VARIOUS TEMPERATURES;
4×100 SEEDS USED IN EACH GERMINATION TEST

| MATERIAL DESCRIPTION | LENGTH OF STORAGE PERIOD | STORAGE TEMPERATURE (°C.) | PERCENTAGE GERMINATION | AT OPTIMUM GERMINATION TEMPERATURE (°C.) | PERCENTAGE LOSS OF VITALITY (°C.) |
|---|--------------------------|---------------------------|------------------------|--|-----------------------------------|
| Hollow Crown ripe, no. 24702, 1924 moisture content 6.33%..... | Fresh | | 88.0 | 20 | |
| | 2 years | 25 | 22.0 | 20 | 75 |
| | 3 years | 25 | None | | 100 |
| Hollow Crown ripe, no. 24266, 1924 moisture content 6.13%..... | Fresh | | 95.0 | 20 | |
| | 6 months | 25 | 97.0 | 20 | None |
| | 2 years | 25 | 60.7 | 20 | 37.5 |
| | 3 years | 25 | 48.0 | 15-32 | 50.6 |
| Hollow Crown green, no. 24702, 1924 moisture content 7.46%..... | 3 years | 5 | 54.0 | 15 | 47.4 |
| | Fresh | | 35.0 | 20 | |
| | 2 years | 25 | 1.7 | | 95.2 |
| | 3 years | 25 | None | | 100 |
| Hollow Crown ripe, Guernsey, 1924 moisture content 6.83%..... | 3 years | 5 | None | | 100 |
| | Fresh | | 95.0 | 20 | |
| | 2 years | 25 | 75.0 | 20 | 20 |
| | 3 years | 25 | 10.0 | 15-25 | 85 |
| | 3 years | 5 | 12.0 | 15 | 83 |

humidity and constant atmospheric moisture are less injurious to seed vitality than lack of ventilation for seeds of ordinary moisture content. There is an indication that the critical moisture content found by HEINRICH (2) for his seeds is somewhat below 6.13 per cent

TABLE VIII
KEEPING QUALITY OF SEEDS WITH REDUCED MOISTURE CONTENT STORED IN
TIGHTLY STOPPED BOTTLES AT VARIOUS TEMPERATURES; 4 X 100
SEEDS USED FOR EACH GERMINATION TEST

| DESCRIPTION OF MATERIAL | TREATMENT | MOISTURE CONTENT | LENGTH OF STORAGE PERIOD | STORAGE TEMPERATURE (°C.) | PERCENTAGE GERMINATION | OPTIMUM GERMINATION TEMPERATURE (°C.) | PERCENTAGE LOSS OF VITALITY |
|---------------------------------------|-----------------------------------|------------------|--------------------------|---------------------------|------------------------|---------------------------------------|-----------------------------|
| Hollow Crown no. 24266, 1924 green | Dried 4 hours 90° C. | 1.27 | None | | 83.0 | 20 | None |
| | | 1.27 | 2 years | 25 | 67.5 | 20 | 18.7 |
| | | 1.27 | 3 years | 25 | 59.0 | 15 | 29 |
| | Dried 24 hours 72° C. C. in vacuo | 0.60 | 3 years | 5 | 70.0 | 15 | 15.7 |
| | | 1.7 | None | | 61.0 | 20 | None |
| | | 1.7 | 2 years | 25 | 76.0 | 20 | Improved |
| | | 1.7 | 3 years | 25 | 75.0 | 15 | Improved |
| | (Dried 1 hour 92° C. | 1.25 | None | | 60.0 | 20 | None |
| | | 1.25 | 2 years | 25 | 51.0 | 20 | 15 |
| | | 1.25 | 3 years | 25 | 39.5 | 15 | 34.2 |
| Hollow Crown ripe, Guernsey 1924..... | Dried 2 hours 92° C. | 1.60 | 3 years | 5 | 47.5 | 15 | 20.8 |
| | | 0.40 | None | | 80.0 | 20 | None |
| | | 0.40 | 2 years | 25 | 69.0 | 20 | 13.8 |
| | Dried 4 hours 92° C. | 0.40 | 3 years | 25 | 50.0 | 15 | 37.5 |
| | | 0.60 | 3 years | 5 | 77.0 | 15 | 3.8 |
| | | 0.40 | None | | 84.0 | 20 | None |
| | | 0.40 | 2 years | 25 | 75.5 | 20 | 10 |
| | | 0.40 | 3 years | 25 | 64.0 | 15 | 23.8 |
| | Dried 6 hours 92° C. | 0.50 | None | | 71.5 | 20 | None |
| | | 0.50 | 2 years | 25 | 73.0 | 20 | None |
| | | 0.50 | 3 years | 25 | 69.0 | 15 | None |
| | | 0.10 | 3 years | 5 | 71.0 | 15 | None |

in ripe parsnip seeds, since seeds with this moisture content keep almost as well as artificially dried seeds of a much lower water content. The keeping quality decreases rapidly, however, with an increase of hygroscopic moisture above 6.13 per cent. Again, green seeds show a greater sensitivity than ripe seeds.

The data in table VIII show that the unfavorable influence of lack of ventilation is avoided if the water content of the seeds is reduced by artificial drying. For green seeds the last method of

storage seems to be the only favorable one. It excludes fluctuating and high atmospheric humidities, to which green seeds have been shown previously to be very sensitive.

The fact that ripe seeds also retain their viability better when

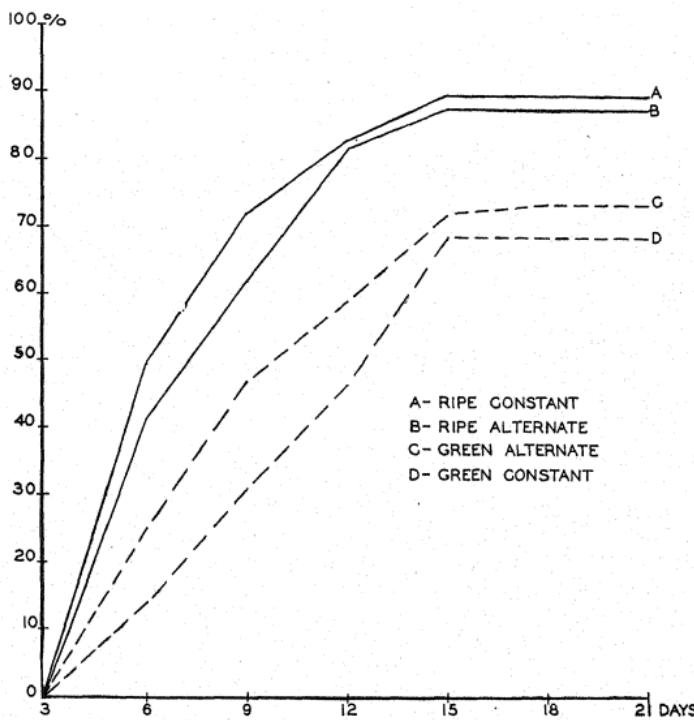


FIG. 1.—Rate of germination in ripe and green parsnip seeds at constant and alternating temperatures immediately after harvest.

stored artificially dried under exclusion of air is shown by a comparison of data in tables VII and VIII.

In addition to the loss in percentage of germination and a shifting in the optimum germination temperature, a slower rate of germination can be noted in stored seeds as compared with freshly harvested materials (figs. 1 and 2).

The germination rate in fresh seeds is highest during the first nine

days and decreases slightly during the following days. Germination is complete after the first fifteen days. The curves for green and ripe seeds are similar in shape, and those for alternating and for constant

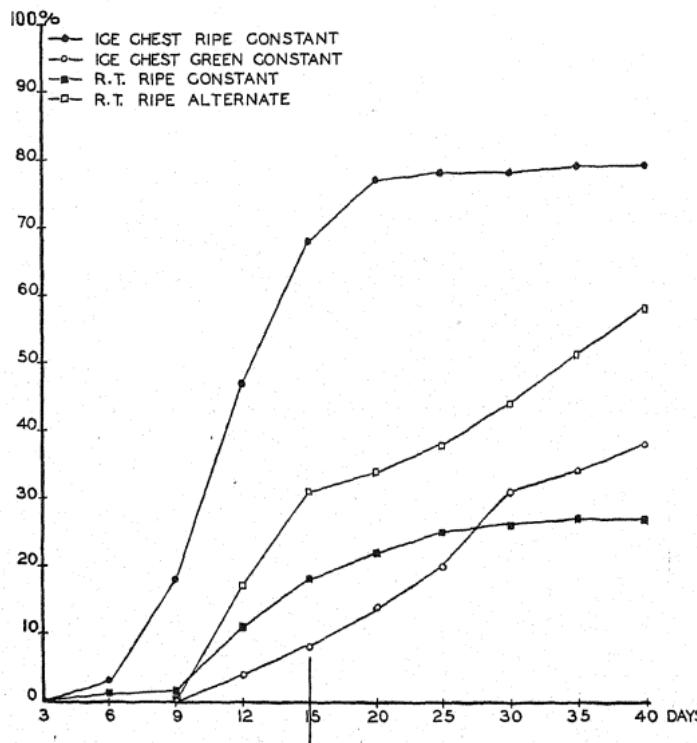


FIG. 2.—Rate of germination in parsnip seeds after three years of storage at room temperature and in ice chest with access of air.

temperatures run close together in both kinds of seeds. After three years of storage, however, curves plotted from similar tests have a very different form. For the first six to nine days the rate of germination is very low, but rises considerably during the following ten days. In seeds of well preserved vitality, such as ripe seeds stored in the ice chest, the optimum percentage of germination is almost reached at the end of this second period, which means that complete germina-

tion is reached after approximately twenty days, five days later than in freshly harvested seeds. In seeds of lower vitality, however, such as ripe seeds stored at room temperature or green seeds, germination proceeds slowly for more than one month, the highest percentage being reached only after forty days. It is also noticeable that the curves obtained from tests with stored seeds lack in regularity as compared with those obtained from experiments with new seeds. This may be due partly to infection by molds, to which stored seeds seem more susceptible than new seeds, but it may also be due to irregularity in absorption of water by stored seeds. In this respect the very low percentage of germination obtained in all stored seeds during the first six days is of special interest. Even seeds of high vitality, such as ripe seeds stored in the ice box, show this decrease in the rate of germination during the first few days. Later the factor which causes this retardation apparently is overcome, and the rest of the curve has the same shape as that for fresh seeds. In seeds of lower vitality, however, the rate remains low throughout the germination period.

Discussion

A comparison of the experimental results reported here with those obtained by MAQUENNE (3) shows that there are several ways of retaining vitality in parsnip seeds besides the one used by MAQUENNE. The writer was able to obtain almost equally good germination (94 and 90.3 per cent) in two consecutive years with parsnip samples of high quality stored under conditions which permit the continuation of metabolic processes (table VI). The writer has also been able to improve the vitality in green seeds during a period of three years (61-75 per cent germination) by storage at reduced moisture content after drying with artificial heat (table VIII). These storage conditions also did not exclude all life processes. It appears, therefore, that parsnip seeds can retain their vitality under certain storage conditions without being transferred to a state of "super-maturation" for at least as long a period as has been used by MAQUENNE in his experiments.

In agreement with the results of DUVEL (1), it was found that temperature and humidity are the main factors to be considered in

parsnip storage; that they are closely dependent upon each other in their effect on seed viability; and that humidity is the more important of the two.

These facts should be taken into account by nurserymen and foresters when they have to store short-lived seed material. At the present time seed growers suffer great losses from an annual discard of seeds which they are unable to sell after the first winter of storage, and foresters find it difficult to hold over a part of their harvest of short-lived fir and pine seeds from one year to another. Since most firs and pines are biennial or periodic bearers, and good harvests are therefore obtained only every second year or at longer periods, it is hard to find enough seed material of good quality for yearly plantings.

With a knowledge of the exact amount of moisture which may be retained in a seed at available storage temperatures without serious injury to seed vitality, it will be much easier for seed growers to store seeds with minimum loss of vitality for several years. For parsnip seeds this "critical moisture content" for ordinary room temperatures has been determined to lie below 6.13 per cent. With a hygroscopic moisture of more than 6.13 per cent the keeping quality of the seeds decreases markedly.

The main symptoms of devitalization, aside from a gradual loss in percentage of germination, are retardation in rate of germination and need of temperature alternations for germination. DUVEL, who has also noticed a decreasing rate of germination in his dry storage material, concludes that the seed coat must become impermeable to water and retard the absorption of moisture necessary to germination. This is not the case in parsnip, however, because the same retardation in germination rate can be observed when the dried seed coats are broken before the seeds are placed in the germination chamber.

Various other possible causes for slow devitalization in dry storage have been suggested by other workers, such as a colloidal rearrangement of substances in the embryo or a gradual denaturing of protoplasmic cell contents, but the experimental proof for these theories has not yet been obtained.

Summary

1. In germination tests conducted shortly after harvest, well-ripened brown seeds give better germination than green seeds.
2. The germination of green seeds can be improved through artificial drying at 60° C. in vacuo for four days.
3. There is no definite optimum germination temperature for freshly harvested seeds, constant and alternating temperatures between 20° and 27° C. being equally favorable.
4. With increasing age, parsnip seeds stored under unfavorable conditions require a temperature alternation of 15° – 25° C. for germination, while seeds stored under conditions favorable to the retention of vitality germinate best at 15° C.
5. Stored in paper bags at room temperature, ripe parsnip seeds lose their vitality at a rate of 20 per cent during the first two years and approximately 60 per cent during three years of storage, when the moisture content of air-dry seeds is 6.33 per cent in the beginning and 5.6 per cent at the end of the storage period.
6. There are various ways by which the keeping quality of seeds may be improved. At a temperature of 5° – 7° C. in an ice box the seeds keep for a considerably longer period, although their moisture content increases in the moist atmosphere of an ice box to more than 1.5 times that of seeds stored at room temperature (9.1 as compared with 5.6 per cent).
7. If the seeds are dried carefully and thoroughly, either for twenty-four hours in vacuo at 72° C. or for four to six hours at 90° C., to a moisture content of 0.40 to 1.7 per cent, their viability remains high even if they are stored at room temperature. To insure a continuous low moisture content, seeds treated in this way have to be kept in air-tight storage. The "critical moisture content" of parsnip seeds lies somewhat below 6.13 per cent.
8. Seeds which are not artificially dried cannot be stored under exclusion of air without losing their vitality very rapidly. Although a low storage temperature retards the death rate of un aerated moist seeds, it does not remove the injurious effect of lack of ventilation.
9. In advising practical storage methods for parsnip seeds, three methods of storing are suggested: (1) Storage at ice box temperature (approximately 5° C.) with frequent stirring of the seeds to secure

good ventilation; with this way of handling, even a very high atmospheric humidity is of little importance to the keeping quality of the seed. (2) When a higher storage temperature has to be used, a thorough drying of the seeds (90° C. for four to six hours) and a subsequent air-tight storage in sealed containers insure good keeping quality. (3) Optimum keeping quality should be obtained where artificially dried seeds are stored air-tight at low temperatures.

BOYCE THOMPSON INSTITUTE FOR PLANT RESEARCH, INC.
YONKERS, N.Y.

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THE
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JOHN MERLE COULTER
(WITH PORTRAIT)

JOHN MERLE COULTER, who founded the BOTANICAL GAZETTE, and who edited it for more than half a century, died December 23, 1928, at Yonkers, New York.

The Gazette in its infancy was a very unpretentious undertaking, consisting each month of a four-page leaflet, the first number of which was issued at Hanover, Indiana, in November, 1875. At first the new journal was known as the Botanical Bulletin, but from the second volume it has been known as the Botanical Gazette, the change of name being made out of regard for the previously existing Bulletin of the Torrey Botanical Club.

For the first few years M. S. COULTER, now better known as STANLEY COULTER, was coeditor with his brother. In 1883 CHARLES R. BARNES, then at Purdue University, and J. C. ARTHUR, then at Charles City, Iowa, became joint editors with Professor COULTER. At that time the journal was much enlarged, and was organized into departments. For many years the Gazette was published by the editors, who bore all financial, as well as editorial responsibility for the journal. At first the subscription price was but \$1.00 a year, and for several years there was no advertising matter, yet almost from the outset the journal paid its way. In the first twenty years of publication the home of the Gazette changed with the abode of one of the editors; at first it was issued from Hanover, Indiana, then from Crawfordsville, Indiana, and Bloomington, Indiana, the suc-

cessive homes of Professor COULTER. From Bloomington it went to Madison, Wisconsin, at that time the residence of Professor BARNES.

In 1896 the *Botanical Gazette* was taken over by the University of Chicago Press, under whose auspices it has since been published. At the same time several prominent American botanists became associate editors, and the following year a group of foreign botanists was added to the list of associate editors. Commencing with 1900, Professor ARTHUR ceased to be a coeditor with COULTER and BARNES, and his name was added to the list of associate editors. Thenceforward the main editorial responsibility rested with Professor COULTER and Professor BARNES, with the assistance of the other members of the botany staff at the University of Chicago. Upon the death of Professor BARNES in 1910, Professor COULTER once more assumed the chief editorial responsibility, and he continued in this capacity until 1926, when the main responsibility passed to the writer of this sketch. Even after the chief editorial burden was set aside, Professor COULTER continued to maintain a lively interest in the journal, contributing many reviews and performing various other editorial functions.

The growth of the *Botanical Gazette* in size and in the number of fields covered in its articles is a measure of the growth of botany in America since 1875. At first the contributions were mostly short taxonomic or floristic notes, without illustrations. Gradually the major contributions lengthened, were more profound in character, and often were illustrated. Morphology was early added to taxonomy as a field of interest, and later contributions appeared in physiology, ecology, mycology, pathology, genetics, and the other fields of modern botany. If Professor COULTER had no other monument than the *Botanical Gazette*, his place in the botanical roll of honor would forever be assured.

JOHN MERLE COULTER was born in Ningpo, China, November 20, 1851, the son of missionary parents. Upon the death of his father in 1853, his mother returned to America with the children, taking up residence at Hanover, Indiana, her father's home. His boyhood days were spent in southern Indiana, mostly at Hanover. He attended Hanover College, from which he was graduated in 1870 with the degree of Bachelor of Arts. His first teaching position was at a

Presbyterian Academy at Logansport, Indiana, where he remained from the autumn of 1870 through the spring of 1872, not as a teacher of botany, but as a teacher of Latin.

In 1872 an event occurred which had a profound influence on Professor COULTER's subsequent career. He was given an opportunity to join the famous Hayden Survey of the Yellowstone, which occupied most of his attention for two years. His appointment was as assistant geologist, but while spending some weeks at Ogden, Utah, where the party was outfitting, he spent his time in collecting and studying the plants of the neighborhood. This came to the notice of Dr. HAYDEN, who needed a botanist, since the originally appointed botanist of the expedition had failed him. So the position was given to Mr. COULTER, who always regarded this incident as a veritable turning point in his life.

The degree of Master of Arts was conferred on the young botanist in 1873 by Hanover College, and from 1874 to 1879 he was Professor of Natural Sciences at that institution. It was during these years that he and his brother founded the *Botanical Gazette*. In 1879 he was called to the chair of Biology at Wabash College, where he remained until 1891. In 1882 he received the degree of Doctor of Philosophy at the University of Indiana, which called him to the botanical professorship and the presidency in 1891. In 1893 he went to the presidency of Lake Forest University. In 1896 he was called to the headship of the new Department of Botany at the University of Chicago, where he remained until his retirement in 1925. In the latter year he took up his residence at Yonkers, New York, the seat of the Boyce Thompson Institute for Plant Research, which he helped to organize, and of whose Board of Directors he was a member from the outset.

Professor COULTER was a charter member of the Botanical Society of America, and was twice its president. He was a long-time member of the American Association for the Advancement of Science and its president in 1919, a member of the National Academy of Sciences, and a member of many other scientific societies at home and abroad.

The early work of Professor COULTER was mostly in the field of taxonomy. This was natural enough, for in the seventies and eighties most American botany was in this field. When he went to Wash-

ton to work up his western collections, he chanced to meet ASA GRAY, who took a great interest in the young Indiana botanist; from that time forth until the death of GRAY in 1888, the friendship between the two was close and intimate. COULTER always attributed much of his success to the influence of GRAY. The Hayden expedition naturally enough suggested the early attention of Mr. COULTER to the flora of the Rocky Mountains, and one of his earliest works was a synopsis of the flora of Colorado, published in 1874 in collaboration with T. C. PORTER. Commencing with 1875, Professor COULTER contributed many articles to the *Botanical Gazette*, the first of which were mainly taxonomic. In 1885 there appeared his well known *Manual of the Botany of the Rocky Mountain Region*, and in 1909 was issued the *Manual of Rocky Mountain Botany* in collaboration with AVEN NELSON. His *Botany of Western Texas* appeared as a contribution of the United States National Herbarium in 1891-1894.

In 1881 Professor COULTER issued, in collaboration with CHARLES R. BARNES, a *Catalogue of the Phaenogamous and Vascular Cryptogamous Plants of Indiana*. Alone or in collaboration Professor COULTER issued a number of taxonomic monographs, as his *Revision of the North American Hypericaceae* in 1886; *Synopsis of the North American Pines* (with J. N. ROSE) in 1886; various works on the Umbelliferae from 1887 to 1909, especially *Revision of North American Umbelliferae* in 1888, *Synopsis of Mexican and Central American Umbelliferae* in 1900, *Monograph of North American Umbelliferae* in 1900, and *North American Umbelliferae* in 1909, all four with J. N. ROSE. In 1894 and 1896 were published works on Cacti. In 1890 was issued the sixth edition of GRAY'S *Manual of Botany*, under the editorship of SERENO WATSON and JOHN M. COULTER.

Early in his career Professor COULTER turned part of his attention to the developing field of morphology, and many outstanding articles and books represent his contribution in this line of endeavor. Perhaps his first contribution in this field was a study of the development of a dandelion flower, published in 1883. Following this was a paper in 1887 on the development of the Umbellifer fruit. But it was after coming to Chicago that his great contributions to morphology were made, mostly in collaboration with CHARLES J. CHAM-

BERLAIN. The most noteworthy of these were the volumes on the Morphology of Spermatophytes, Morphology of Angiosperms, and Morphology of Gymnosperms, published respectively in 1901, 1903, and 1910. Other morphological contributions were issued in the form of papers on the Phylogeny of Angiosperms in 1903, the Embryogeny of *Zamia* (with C. J. CHAMBERLAIN) in 1903, Development of Morphological Conceptions in 1904, Gametophytes and Embryo of *Torreya taxifolia* (with W. J. G. LAND) in 1905, Relation of Megaspores to Embryo Sacs in Angiosperms in 1908, Embryo Sac and Embryo of *Gnetum gnemon* in 1908, Evolutionary Tendencies among Gymnosperms in 1909, An American *Lepidostrobus* (with W. J. G. LAND) in 1911, the Endosperm of Angiosperms in 1911, and the Origin of Monocotyledony (with W. J. G. LAND) in 1914.

In the field of botanical textbooks Professor COULTER made rich contribution. In 1886 appeared a Handbook of Plant Dissection by ARTHUR, BARNES, and COULTER, often familiarly called a botany ABC. Plant Relations appeared in 1901, Plant Structures in 1904, Plant Studies in 1904, Textbook of Botany in 1906, and Elementary Studies in Botany in 1913. Professor COULTER wrote the morphological section of the more advanced Textbook of Botany, issued in 1910 in collaboration with CHARLES R. BARNES and HENRY C. COWLES.

Professor COULTER contributed also to fields other than those just noted. In 1914 he issued a book on the Fundamentals of Plant Breeding, and a book on the Evolution of Sex in Plants; and in 1916 a book on Evolution, Heredity, and Eugenics. He published from time to time papers on evolution, on various educational and religious subjects, and on topics dealing with the relation between science and religion.

Great as were Professor COULTER's contributions to taxonomy, morphology, and other fields, and as a botanical editor, it is probable that his greatest single influence was as a teacher. He was an inspiring lecturer, a splendid counselor, and a devoted friend. His kindly sympathy and help drew all his students closely to him, and made them devoted followers. He inspired many men and women to devote themselves to botanical research and botanical teaching.

This was true at Hanover, Crawfordsville, Bloomington, and Lake Forest, but it was particularly true at Chicago, for there as nowhere else in his previous experience came opportunity to teach, influence, and inspire graduate students from colleges and universities from this and other countries. During his headship at Chicago 175 students attained under him the degree of Doctor of Philosophy, and almost as many attained the degree of Master of Science. Shortly after coming to Chicago he initiated the series of researches known as "Contributions from the Hull Botanical Laboratory." These include contributions made by Professor COULTER and other members of the Chicago botany staff, nearly all of the Doctor's theses, and many of the Master's theses. Nearly 400 of these have been issued in the Botanical Gazette.

The students of Professor COULTER have more than once shown their appreciation of their leader. In 1916, at the occasion of the quarter centennial of the University of Chicago, the botany doctors, then 80 in number, presented to him a volume giving the record of the doctors to that date. On December 27, 1928, at New York, there culminated a movement to establish at Chicago a JOHN M. COULTER Research Fellowship in Botany. This movement had been initiated by the Chicago botany doctors two years previously at Philadelphia, and at New York it was announced that pledges amounting to more than \$25,000 were in hand, thus assuring the fellowship. This fund was subscribed by 130 doctors and 75 masters and former students of Professor COULTER. It is expected that the first fellowship on this foundation will be available in the academic year 1929-1930. Almost simultaneously with the announcement of the COULTER Fellowship, there was presented to Mrs. COULTER a silver service and a volume of testimonials to Professor COULTER from the botanists of America. It had been the hope that Professor COULTER would be present in person to hear the announcement of the Fellowship and to receive the silver service and the volume of testimonials. It was a tragic coincidence that he died but a few days before these events were scheduled to take place. It is, however, a matter of satisfaction that before he died he was apprised of both events and was highly gratified because of them.

A scientific journal, such as the *BOTANICAL GAZETTE*, is hardly the place to speak more intimately and appreciatively of Professor COULTER's life and influence, even though this is the journal that he founded and edited for so many years. It is perhaps enough to say that there has passed from us a man loved and admired, not only by his fellow botanists and former students, but also by many in other fields of science, and in every walk in life; that there has gone a great teacher, a gifted editor, an inspiring lecturer, and a facile writer; and that, as he himself said on the death of Professor BARNES, "a priceless asset has become a memory."—H. C. COWLES.

MEIOSIS IN POLLEN MOTHER CELLS OF STRAINS
OF *OENOTHERA PRATINCOLA* BARTLETT^x

CHANDRAKANT G. KULKARNI

(WITH PLATES VII-IX)

Introduction

Investigations of the nuclear behavior in the pollen development of *Oenothera lamarckiana* and certain of its derivatives, together with some of the other large-flowered, generally cross-pollinated species of *Oenothera*, have made clear some of the reasons for the peculiar genetical behavior that characterizes this genus, even though all the hypotheses proposed are in some respects at variance with one another. So far, few cytological investigations of the small-flowered, generally self-pollinated species have been made. There are papers by DAVIS (23), EMERSON (32), CLELAND (18, 19), and VALCANOVER (88); there are no cytological publications whatever on the types studied genetically by BARTLETT, FRIEDA COBB BLANCHARD, LARUE, KLAPHAAK, and MARAÑON at the University of Michigan. Of these, *O. pratincola* is the foremost in interest, due to the fact that it has been most intensively studied and has given rise to the most mutations. In addition to many genetical phenomena that are common with the large-flowered species, *O. pratincola* shows behavior peculiar to itself and to other small-flowered species (LARUE and BARTLETT 56, 57).

The strains of *O. pratincola* used in this research are those whose history has been published by BARTLETT (2, 3, 5) and COBB and BARTLETT (20). Although the eight strains of this species from Lexington, Kentucky are morphologically alike, the strain designated E has a genetical behavior differing from the other seven. This strain gave rise to variants in such large numbers that BARTLETT (4, 5) designated the phenomenon mass mutation. Some of the mutations produced by the other strains are also produced by strain E, but the latter is peculiar in throwing a series of forms with narrow,

^x Papers from the Department of Botany of the University of Michigan, no. 298. Walker prize essay of the Boston Society of Natural History, 1928.

strongly revolute, thick-veined leaves, which do not occur in the other strains. *Mut. formosa*, an example in this series, has been completely described and illustrated (BARTLETT 5). *Mut. formosa* was chosen for this study because, being the most fertile of the revolute-leaved forms, it was most successfully used in crosses of genetical and cytological significance for the problems under discussion.

Oenothera pratincola strain C gives rise to mutations of several kinds in every generation (BARTLETT 3). This strain differs from strain E in that it has never shown mass mutation since coming under cultivation. Strain C is typical of the seven others, and was chosen for crossing with strain E and also with *mut. formosa*.

Strain M comes from a cross between *mut. formosa* and strain C, the former being the female parent. It was designated "M" because it shows Mendelian segregation. When *mut. formosa* is pollinated by strain C, the F_1 plants are all *f. typica*, that is, similar to *O. pratincola*. The F_2 generation splits into 3 *f. typica*: 1 *mut. formosa* (COBB 21). This strain becomes of great cytological and genetical interest because simple cases of Mendelian inheritance in *Oenothera* are uncommon. The first described case of Mendelian inheritance is afforded by *O. brevistylis*, which acts in crosses as a recessive with *O. lamarckiana* and even with unrelated species (DE VRIES 73, DAVIS 25, 26). The *brevistylis* complex, when introduced into the form *nanella-brevistylis*, is also recessive; when this plant is crossed to *nanella* and to *lamarckiana* it segregates in the F_2 in proportions close to the 1:3 Mendelian ratio (DAVIS 27). Another example showing Mendelian behavior is the dwarf mutation from *O. gigas* which also acts as a recessive in crosses with its parent (DE VRIES 92). It has been the contention of GATES (44, 45) that *O. rubricalyx* acts as a Mendelian dominant in crosses with *O. rubrinervis*, but SHULL (77) offers another interpretation. Another character, "old-gold," a flower color which arose as a gene mutation from *O. lamarckiana*, has recently been added by SHULL (80) to the list. In crosses with its parent *O. lamarckiana*, which has yellow flowers, this character acts as a Mendelian recessive.

Material and methods

The material on which this research is based was collected from pedigree cultures in the Botanical Garden of the University of

Michigan, during the summers of 1925 and 1926. Various fixing fluids were used, of which only one (weak Flemming) failed entirely to give satisfactory results. One of Allen's modification of Bouin's solution (saturated aqueous solution of picric acid 75 cc., commercial formalin 25 cc., glacial acetic acid 5 cc., chromic acid 1 gm., and urea 2 gm.) gave excellent results. Allen's modification of Bouin's solution as used by CLELAND (16) was also very good. Strong Flemming with 1 per cent urea and 1 per cent maltose added, yielded very satisfactory fixations, as good as those of Allen's modification of Bouin's. Straight Bouin's solution also proved excellent. The fixations in chromo-acetic solution containing chromic acid 1 gm., glacial acetic acid 3 cc., and distilled water 200 cc. were generally good. When using strong Flemming it is necessary to wet and scrub the anthers quickly with a stiff hair brush, as suggested by DAVIS (23, 24), so as to loosen the waxy coat that envelops them, and thus facilitate quick penetration by the killing fluid. If this procedure is not followed with great patience and care, the fixations in strong Flemming are likely to be generally very poor.

Twelve fixations were made from four plants of *O. pratincola* strain E, six from three plants of *O. pratincola* mut. *formosa*, nine from three plants of *O. pratincola* strain C, and eight from four plants of *O. pratincola* strain M. Each fixation consisted of twenty-five buds.

Whenever a fixation was secured from a plant, pollen counts were made on four or five buds, picked at random, to determine the percentage of good pollen. The results of these tests are included in table I, and show for the four strains good pollen averaging about 86 per cent. Table I also gives the time at which fixations were made, the length of time the buds were left in the fixing fluid, the kind of fixing fluid used, and the results from various fixations.

When strong Flemming solution was used, the buds were left in the solution for two to three hours, and were then transferred for 24 hours to chromo-acetic solution, to avoid the excessive blackening of the material that ensues from the action of the osmic acid. In case of Bouin's solution and its modifications, three to six hours in the fluid gave excellent results.

All the stages of cell division were plentifully obtained in the

majority of fixations, made between 11:30 A.M. and 3:30 P.M. The material was dehydrated through grades of alcohol, cleared in xylol, and imbedded in paraffin. Longitudinal sections, varying from 6 to

TABLE I
QUALITY OF FIXATION AND TIME OF FIXING; POLLEN CONDITION OF PLANT

| CULTURE | STRAIN | TIME OF DAY | HOURS IN FIXING FLUID | FIXING FLUID | QUALITY OF FIXATION | POLLEN GRAINS COUNTED | PERCENTAGE GOOD POLLEN* |
|----------|----------|-------------|-----------------------|-----------------|---------------------|-----------------------|-------------------------|
| 68.1... | E | 3:30 P.M. | 3 | Bouin modified | Excellent | 350 | 90.3 |
| 68.9... | E | 11:30 A.M. | 4 | " " | " | 492 | 91.9 |
| 68.9... | E | 12:30 A.M. | 7 | " " | " | 543 | 85.2 |
| 68.1... | E | 10:45 A.M. | 6 | " " | " | 137 | 74.3 |
| 68.1... | E | 1:45 P.M. | 6 | " " | " | 260 | 85.0 |
| 68.9... | E | 1:30 P.M. | 5 | Bouin | Good | 129 | 84.9 |
| 68.4... | E | 1:45 P.M. | 28 | Chromo-acetic | " | 490 | 86.0 |
| 68.4... | E | 12:30 P.M. | 24 | Strong Flemming | Excellent | 114 | 82.5 |
| 68.1... | E | 10:00 P.M. | 24 | " " | " | 168 | 92.3 |
| 68.1... | E | 2:00 A.M. | 8 | Bouin modified | Very good | 182 | 81.9 |
| 68.1... | E | 12:00 A.M. | 7 | " " | Excellent | 351 | 92.4 |
| 68.5... | E | 2:45 A.M. | 26 | Weak Flemming | Very good | 168 | 86.4 |
| 63.6... | For-mosa | 11:35 A.M. | 5 | Bouin modified | Excellent | 220 | 89.5 |
| 63.8... | " | 11:00 A.M. | 7 | " " | " | 645 | 89.2 |
| 63.8... | " | 4:15 P.M. | 3 | " " | " | 320 | 87.4 |
| 63.6... | " | 3:40 P.M. | 4 | " " | " | 214 | 82.5 |
| 63.7... | " | 1:45 P.M. | 5 | " " | " | 113 | 83.5 |
| 63.7... | " | 10:15 A.M. | 7 | " " | " | 108 | 83.9 |
| 610.3... | C | 12:20 A.M. | 6 | Bouin modified | " | 64 | 93.4 |
| 610.3... | C | 3:00 P.M. | 24 | Strong Flemming | " | 78 | 76.2 |
| 610.3... | C | 12:10 A.M. | 6 | Bouin modified | " | 213 | 93.5 |
| 610.3... | C | 11:45 A.M. | 24 | Strong Flemming | " | 420 | 91.8 |
| 610.4... | C | 4:20 P.M. | 24 | " " | " | 205 | 82.9 |
| 610.3... | C | 1:00 P.M. | 6 | Bouin modified | " | 94 | 78.0 |
| 610.3... | C | 1:35 P.M. | 5 | " " | " | 150 | 84.7 |
| 610.1... | C | 12:45 P.M. | 24 | Weak Flemming | Very poor | 108 | 92.0 |
| 69.4... | M | 11:35 A.M. | 5 | Bouin modified | Excellent | 345 | 91.2 |
| 69.4... | M | 4:30 P.M. | 3 | " " | " | 219 | 90.5 |
| 69.4... | M | 2:30 P.M. | 5 | " " | " | 235 | 93.2 |
| 69.4... | M | 12:55 P.M. | 6 | " " | " | 400 | 90.1 |
| 69.2... | M | 1:10 P.M. | 7 | " " | " | 139 | 63.0 |
| 69.5... | M | 1:20 P.M. | 4 | " " | " | 321 | 87.8 |

* Average percentage of good pollen in strain E was 85.2; in mut. *formosa*, 86.00; in strain C, 86.5; and in strain M, 85.8.

11 μ , were cut and stained in iron-alum haematoxylin, which proved excellent for the sharp differentiation of the nuclear structures. All the important and critical stages in the development of the pollen were plentifully obtained from the various individuals from which the fixations were made.

MEIOSIS IN *O. PRATINCOLA* STRAIN E

MITOSES IN ARCHESPORIUM OF ANTER.—The mitoses in the archesporium of the anthers of *Oenothera pratincola* strain E, conform generally to the account of *O. grandiflora* Solander given by DAVIS (22), and hence only the critical stages will be discussed.

Differentiation of the archesporium in the anthers of *O. pratincola* takes place at a very early period. Cells divide both lengthwise and crosswise during its development, and finally a single and sometimes a double row becomes transformed into pollen mother cells. Numerous nuclear divisions take place at the same time in various regions of the anther.

The sporophytic mitosis is best studied in the final mitoses of the archesporial cells, which generally convert the primary single row of cells into a double row that later becomes the pollen mother cells. The somewhat greater size of the nuclei, as compared with the nuclei of the other cells of the developing flower, facilitates accurate observation.

The diploid chromosome number in *O. pratincola* strain E and *mut. formosa* is fourteen, as usual for species of *Oenothera*. This can be seen clearly in the nucleus before the appearance of the spindle fibers prior to the metaphase of the last division in the archesporium. The chromosomes at this time appear as variously bent rods, as shown in the polar view of the equatorial plate presented in fig. 1. During the metaphase and the early anaphase the chromosomes assume the forms of thick U's and V's, due to condensation (fig. 2). In the late anaphase and with the approach of telophase, condensation proceeds still further. Because the chromosomes are so closely massed during the stages of late telophase, it was impossible to determine whether they undergo a definite lengthwise split into two parallel threads, as claimed by DIGBY (30) for *Osmunda* and FRASSER and SNELL (35) for *Vicia faba*, or whether they become irregularly alveolized and form together a continuous reticulum, as described by SHARP in *Vicia* (75) and *Tradescantia* (76). The nuclei then pass into the resting condition preliminary to meiosis, in which the outlines of individual chromosomes become entirely lost.

RESTING NUCLEUS BEFORE MEIOSIS.—The nuclei of the pollen mother cells pass through a relatively long period of rest before the

advent of the prophase of the heterotypic division. They measure 9-11 μ , and possess a prominent nuclear membrane. The most conspicuous structures in the nucleus at this time are the nucleoli, frequently three or four in number and sometimes as many as five and six. Only one of these bodies attains a large size (fig. 3), however, and sometimes a shining area resembling a vacuole may distinctly be observed within it. The large nucleolus is nearly spherical, and lies toward the center of the nucleus. The smaller nucleoli are scattered in the nucleus, and seem to have no definite positions. It is difficult to determine the exact number of nucleoli, since some of them are as small as the larger chromatin granules. Certain of the smaller nucleoli are frequently seen lying beside the largest, and these probably unite with the latter, as reported by GATES (43) in the case of *O. rubrineris*.

During the resting stage the nucleus contains a chromatic reticulum of delicate single threads, evenly distributed through its interior (fig. 3). Very small chromatin granules may be seen irregularly scattered over these threads. At the juncture of two threads chromatin granules of varying shapes and sizes were distinctly observed. These fine threads run through peripheral regions of the nucleus and over the surface of the nucleolus, and are in direct contact with the chromatic material in the nucleolus (fig. 3). The threads are uniformly single and show no parallelism.

PRESYNTHESIS STAGES.—Shortly before the resting nuclei enter the prophase of the heterotypic mitosis, some parallelisms in the threads of the reticulum may be observed, but these are not numerous and seem not to be significant. It is quite natural that some parallelisms among the threads should exist, taking into consideration their number within so small a space. At the approach of the heterotypic prophase the threads of the reticulum begin to thicken and to contract gradually, and many of them unite with one another in an irregular manner. This process transforms the characteristic fine-meshed reticulum of the resting stage (fig. 3) into an open reticulum, the threads of which are coarser and rougher (figs. 4, 5). The union of threads with subsequent condensation which effects the transformation of a delicate reticulum into a system of coarse and rough threads, varying in diameter, is a gradual process (fig. 6).

While this is taking place, there may still be observed in the nucleus some delicate threads not yet thickened; and also some shriveled threads may be observed which appear to be in the process of delivering their chromatin content to the threads that are to survive. The chromatin accumulations that are scattered irregularly throughout the nucleus during the resting stage probably become amalgamated with the thickening threads, and even if they exist their presence is not recognized with certainty. The pull exerted on the peripheral threads, due to the contraction, shrinkage, and condensation of the internal threads, causes them to draw away from the nuclear membrane and they come to lie in a tangled mass at one side of the nucleolus, giving the characteristic stage of synizesis. Occasionally the threads very close to and connected with the nuclear membrane may still be seen in place, but they are not smooth and continuous (fig. 6). The nucleolus leaves its central position in the nucleus and moves to the periphery, where it generally lies flattened against the nuclear membrane, next to the contracted mass of threads (fig. 7).

Some parallelism among the threads may be observed at times during this process of condensation; but since the process is one of contraction, it is to be expected that threads will occasionally be drawn into side-by-side relations which are not to be regarded as significant.

The transformation of the fine-meshed reticulum, composed of single delicate threads with scattered chromatin granules of varying shapes and sizes, into a reticulum of rather large meshes, composed of threads of coarser and rougher nature, forming a spireme, has given rise to much discussion among prominent cytologists as to the method by which this transformation is brought about. Two interpretations have been offered for various material. One is supported by GRÉGOIRE (50, 51), ALLEN (1), ROSENBERG (69, 70, 71), STOMPS (85), and others and the other interpretation is held by FARMER and MOORE (33, 34), DIGBY (28, 29, 30), MOTTIER (65, 66, 67), FRASER and SNELL (35), FRASER (36), and their followers. Both schools agree that the most important event of this process in most material is a side-by-side association of threads during the earlier part of the prophase, but the two schools offer very different inter-

pretations of the history. The first interpretation, that of para-synapsis, holds that the two threads are homologous spiremes, one derived from the egg and the other from the sperm. These threads hold two sets of chromosomes, and their association gives rise to a bivalent spireme. The second interpretation, that of telosynapsis, maintains that the double thread when present is due to the length-wise division of chromosomes during telophase, or even anaphase of the previous mitosis, and consequently does not represent an association of two spiremes of different descent. By the interpretation of telosynapsis, double threads, if found during the prophase, are merely associations of daughter spiremes.

The history of meiosis in *Oenothera pratincola* and all its strains favors the telosynaptic interpretation.

SYNIZESIS

The thickening of the threads goes on for some time, due to contraction, and also because material is received from the chromatin granules and the other threads (fig. 6). Some of the threads become very thin, and certain of them become quite shriveled as their material presumably passes into the developing spireme. Certain parts of the threads near the nucleolus become gorged with material, appearing much swollen. Material from the nucleolus is apparently received by the nearest threads more rapidly than it is dispersed, and consequently parts of the threads become much thickened. The reticulum at this stage (fig. 6) shows a spireme composed of rather thick and uniform threads, some of which may be followed for a considerable distance in the tangled mass. The nucleolus loses its spherical shape and becomes somewhat flattened against the nuclear membrane. It is quite empty of chromatic material, as is seen by its reaction to the stain; but the endonucleolus is clearly seen as a very small round body taking the stain conspicuously (fig. 9). The spireme becomes more closely contracted, and finally has the appearance of a closely wound ball of threads, the synizetic knot (fig. 8). The knot remains in this much contracted condition of mid-synizesis for a long time, while the threads of which it is composed become more uniform in thickness and longer. The process of transformation from a reticulum of delicate threads into the spireme

that emerges at the end of synizesis takes place very slowly; in fact, synizesis is of longer duration than any other period in the development of the pollen. During certain stages the knot becomes so dense that it is impossible to determine with certainty what is happening within.

OPEN SPIREME

After some time the knot gradually begins to loosen, probably due to the lengthening of the threads. Many small loops of various sizes begin to extend from the periphery of the knot, while the greater portion of it is still so contracted that nothing of its structure can be distinguished with accuracy (fig. 9), although it is clear that it is composed of thicker threads. The loops on the periphery of the knot loosen while the central mass of threads is still drawn together in a very complicated manner (fig. 10). Sometimes during this stage the threads are so thick and full of chromatin that it is very hard to recognize them as threads. Finally they become uniform and those in the center loosen sufficiently to reveal their individuality. During no stage is the spireme entirely clear and free from chromatin accumulations, however, but differential staining shows it to be composed of distinct threads much swollen and gorged with material (fig. 11). During this emergence of the spireme from the contracted condition of mid-synizesis the threads become distributed uniformly throughout the nucleus. Some of them become very prominently beaded, the beads being placed in a single row, and these are probably the chromomeres of various authors (fig. 12). It is possible to trace a single thread for some distance as it winds about in the nucleus. No threads with free ends were found in this stage.

The nucleolus which lies flat against the nuclear membrane throughout synizesis and during the emergence of the open spireme later takes on a spherical form, and generally moves to the center of the nucleus. It is quite homogeneous and free from chromatic material, as indicated by its failure to stain; but the minute, deep-staining endonucleolus within may be distinctly observed (figs. 12, 13, 15).

The spireme has been carefully studied for evidence of double structure but none has been found; the threads are everywhere uniformly single.

During synizesis and the stages of the open spireme the pollen mother cells become round and separate from one another. In the stages prior to synizesis they are packed closely together and consequently are angular.

SECOND CONTRACTION

After remaining for some time in the open spireme stage, the nucleus enters the stage known as the second contraction. This stage is of relatively short duration. The threads that were rather widely and evenly distributed during the open spireme stage begin to contract, with accompanying condensation of chromatic material. The process of contraction and condensation takes place rapidly, and consequently the threads at the periphery come to form radiating loops from the central mass (fig. 13). The threads in the center become much swollen and form large irregular masses of chromatin (figs. 14, 15). The individual threads appear to be lost in the central mass, but differential staining shows this chromatic mass really organized, consisting of closely contracted and much thickened threads (fig. 13). It was not possible to determine whether these central threads unite with one another as they come to lie in a mass, but close observation indicates that they are distinct and separate, although very much swollen (figs. 14, 15).

As condensation proceeds, the material from the radiating loops is drawn to the center, and at the time of the greatest contraction the contents of the nucleus look like an irregular mass of chromatin with very slight traces of loops at the periphery. In no case, however, are these loops lost during any stage of second contraction. As the loops become shorter and shorter their two sides approach, until in some cases they actually come to lie against each other. Very soon there appear constrictions in the loops, which probably indicate segments of the spireme that are to become the chromosomes. Some loops are made up of only one chromosome (fig. 14), while others may have as many as two or three (fig. 15). After some time the outlines of the chromosomes become clear. While some of them may be distinguished clearly in one part of the contracted mass, it frequently happens that in other parts definite organization cannot be recognized (fig. 15). The formation of the chromosomes through segmentation of the spireme is easily demonstrated.

A most careful study failed to show any evidence of lengthwise fission among the loops in the spireme during the second contraction; the spireme was found to be univalent in nature, the threads being everywhere single. A split in the spireme would be expected if it were bivalent, since it is at this stage that the chromosomes prepare to separate from each other; therefore it is certain that each of these loops represents one or more chromosomes attached end to end.

The nucleolus, during the stage of second contraction, generally lies against the nuclear membrane, and is oval in form with little or no stainable material. In some nuclei it comes to the center. The endonucleolus may be observed at this stage as a black body inside the nucleolus (figs. 13, 15), which is not at all involved in the processes of second contraction.

FINAL PROPHASE STAGES

Ordinarily in plants and animals the term diakinesis is used to denote the stage when the paired homologous chromosomes lie in the nucleus prior to the formation of the heterotypic spindle. It is doubtful whether this term should be used at all in the case of *Oenothera pratincola*, which shows no pairing but a closed chain of fourteen chromosomes attached end to end. During the prophase stages so far discussed there is little opportunity for studying the individual chromosomes, because of the complexity of the stages through which they pass, and also due to the fact that they are not in a compact form; but from now on stages will be discussed in which individual chromosomes are clearly shown.

At the end of the second contraction there emerge gradually chromosomes, irregular in outline and connected end to end by delicate threads. They sometimes lie over one another in a confused mass, and frequently their arrangement cannot be determined with accuracy (fig. 16). Later they become more evident with the loosening of the contracted mass (fig. 17). Shortly after this stage the whole situation clears, and a very striking arrangement of the fourteen chromosomes attached by delicate threads end to end in a closed chain is readily observed (figs. 18-20). Sometimes an attachment breaks and instead of a closed chain of fourteen chromosomes an

open chain results (fig. 21). It is difficult, because of the length of the chain of chromosomes and because of its loops, to find sections in which all the members are present, all the attachments undisturbed and perfectly clear. I have examined nearly one thousand cells in this species and found no case of chromosomes in a paired side-by-side arrangement. The results of a critical examination of the nuclei at this stage are summarized in table II. Although many nuclei had been cut, so that certain chromosomes were missing,

TABLE II
ARRANGEMENT OF CHROMOSOMES DURING LATE PROPHASE IN
OENOTHERA PRATINCOLA

| ARRANGEMENT | NUMBER OF NUCLEI OBSERVED |
|---|---------------------------|
| Closed chain of fourteen chromosomes | 37 |
| Open chain of fourteen chromosomes (attachment broken at one point) | 17 |
| Total | 54 |
| Thirteen chromosomes in chain, one missing | 19 |
| Twelve chromosomes in chain, two missing | 31 |
| Eleven chromosomes in chain, three missing | 44 |
| Ten chromosomes in chain, four missing | 58 |
| Nine chromosomes in chain, five missing | 35 |
| Eight chromosomes in chain, six missing | 30 |
| Total | 217 |
| Total | 271 |

the observations uniformly showed that all the chromosomes present were in a continuous chain or ring.

This final stage of the heterotypic prophase lasts only for a short time. The nucleolus, pressed against the nuclear membrane, quite free of the chromatic material, disappears slowly. The deep-staining endonucleolus persists even after the disappearance of the nucleolus (fig. 23).

HETEROTYPIC METAPHASE

The nuclear membrane breaks down slowly. Its dissolution is first indicated by the surrounding cytoplasm becoming rather dense and by the development of delicate fibrils. The fibrils are at first scattered without order. When the nuclear membrane has complete-

ly dissolved they appear to penetrate the nuclear cavity, and very soon a multipolar spindle is formed with the fibers arranged in cones (fig. 23). The fibers enter the cluster of chromosomes in an irregular and complicated manner. The chromosomes, which have an irregular outline during the final stages of prophase, change in shape and size with condensation, becoming quite compact, when they look like thick V's and rods. The closed chain of fourteen chromosomes still persists (fig. 22), except when occasionally a delicate attachment breaks and an open chain results (fig. 23). The endonucleolus may be observed even at this stage, but with the approach of anaphase it disappears.

The multipolar spindle becomes bipolar, and the chromosomes, still in a closed chain, pass to the equatorial region of the spindle. Shortly after, the spindle fibers, which by this time are organized into clusters, attach themselves to the mid-region of the V-shaped chromosomes in such a way that the alternate chromosomes go to the same pole (fig. 24). Some of the chromosomes are rod-shaped, in which case the spindle fibers are attached to their ends. In view of the fact that all the fourteen chromosomes are attached to one another end to end in a chain, which is sometimes very much looped, it is difficult to obtain a clear view of all the chromosomes and their attachments. In spite of these difficulties, a thorough study was made of the best nuclei at this stage, so as to determine the exact nature of the attachments of the spindle fibers to the chromosomes. Only the nuclei presenting a clear view of all the fourteen chromosomes with their attachments have been included in this survey, a summary of which is given in table III. Hundreds of cells have been examined in which some of the chromosomes were missing but the attachments present were intact; these have not been included in table III.

The data presented in table III clearly show that the arrangement of the chromosomes in many nuclei during the heterotypic metaphase is zigzag. As a result of such an arrangement, alternate chromosomes go to the same pole in the species under discussion. Certain irregularities in the zigzag arrangement do occur, however, the percentage of which is greater in this strain than in *O. biennis* and "*O. muricata*," as reported by CLELAND (18, 19). It is 30.1 per cent for *O. pratincola* strain E. The irregularities may conveniently

be listed under the following headings: (1) Two chromosomes in different parts of the chain are suspended between the poles (fig. 25 *a, b*). If both of these go to the same pole, the distribution will be eight chromosomes to one nucleus and six to the other. In case they go to different poles the distribution will be seven chromosomes to each nucleus. (2) Two adjacent chromosomes in one part of the chain are attached to the spindle fibers leading them to the same pole; and in the other part of the chain a corresponding pair is similarly attached to fibers that lead them to the other pole (fig. 26 *a, b*). In

TABLE III
RESULTS OF SURVEY OF CELLS DURING HETEROGENETIC METAPHASE
SHOWING CHROMOSOME ARRANGEMENT

| CHROMOSOMES ALL DISTINCTLY VISIBLE AND END-TO-END ATTACHMENT CLEAR | NUMBER OF NUCLEI OBSERVED |
|---|---------------------------------|
| Closed chain of fourteen chromosomes complete and regularly zigzag..... | 135 |
| Open chain of fourteen chromosomes complete and regularly zigzag..... | 39 |
| | <hr/> |
| Total | 174 |
| | <hr/> |
| Closed chain of fourteen chromosomes complete and attachments irregular..... | 43 |
| Open chain of fourteen chromosomes complete and attachments irregular..... | 32 |
| | <hr/> |
| Total | 75 |
| | <hr/> |
| Total | 249 |

this type of irregularity, however, the number of chromosomes going to the two poles will be equal, seven to each pole. (3) A pair of adjacent chromosomes is drawn to the same pole, while a chromosome is uncertainly suspended in the equatorial region (fig. 27 *a, b*). This chromosome might go to either pole. If it is drawn to the pole with the abnormally placed pair, the number of chromosomes passing to that pole will be eight and the other pole will then have only six chromosomes. All these irregularities have been noted by CLELAND (18, 19) for *O. biennis* and "*O. muricata*." (4) Three or sometimes four chromosomes in a group have fiber attachments of such a nature that it is impossible to predict the number that might go to the two poles (figs. 28-30).

Careful observation during mid-interkinesis following the hetero-

typic mitosis also gives some idea of the irregularity in the chromosome distribution. Nine hundred nuclei in this stage were examined, of which ninety-four were found to have eight or six chromosomes, which is an irregularity of 9.4 per cent. Since irregularity in the zig-zag arrangement during metaphase is not necessarily the only cause of abnormal distribution (six and eight) of the chromosomes, the total percentage of irregularities is higher, and was actually found to be about 30.1 per cent.

HETEROTYPIC ANAPHASE AND TELOPHASE

In early anaphase the chromosomes present the zigzag arrangement (fig. 24) which is so clearly described and illustrated by CLELAND in all his papers on the cytology of *Oenothera*. The chromosomes separate during anaphase into two sets, breaking their connection with each other. Presently they reach the two poles of the spindle, seven passing to each pole when the distribution is regular (fig. 31), and eight to one pole and six to the other if the distribution is irregular (fig. 32). Soon after the chromosomes reach the pole they generally form a small star-shaped cluster, with one chromosome in the center and six at the periphery, an arrangement typical of species of *Oenothera* at this stage (fig. 33). After some time the chromosomes split lengthwise (fig. 34), the split at first not being conspicuous. The chromosomes spin out delicate threads which generally connect them with one another. A vacuole-like space appears around the group of chromosomes, and the outer boundary of this area marks the nuclear membrane.

INTERKINESIS

The slender threads connecting the chromosomes become hazy during the interkinesis and the nuclei enlarge rapidly. The chromosomes also enlarge and become irregular in form. Shortly after this the split in the chromosomes becomes conspicuous, and the halves frequently separate to give an irregular X (fig. 35). These halves or daughter chromosomes are loosely connected in their middle region. In certain cases the chromosomes come to show denser regions within, which stain more deeply than the main body of the chromosomes (fig. 35).

During this stage one or more nucleoli appear at first as minute globules, in connection with chromosomes. Sometimes they are associated with the chromatin threads that pass between the chromosomes (fig. 34). It appears that they arise *de novo*, as there is nothing to indicate their survival from previous stages.

HOMEOTYPIC DIVISION

The daughter chromosomes which arise from the lengthwise division of the heterotypic chromosomes condense during the pro-phases and finally appear as short rods (fig. 36). As soon as the nuclear membrane breaks down, the spindle fibers become conspicuous and enter the nuclear cavity. Very soon a multipolar spindle is formed, which later becomes bipolar, and the chromosomes pass to the equatorial region of the spindle (fig. 37). The fibers, which are organized by this time into thick clusters, attach themselves to the perfectly paired daughter chromosomes. Shortly after this the daughter chromosomes separate and pass to the poles, seven chromosomes in each set. The homeotypic division is normal except where there has been an irregularity in the preceding heterotypic mitosis, in which case eight and six chromosomes respectively may be distributed instead of the normal seven (fig. 36).

The four groups of chromosomes resulting from the homeotypic division lie rather close together in the pollen mother cell. Shortly, a clear hyaline space resembling a vacuole arises around each group. The outer boundary of this vacuole ultimately becomes the nuclear membrane. The nuclei then enlarge rapidly, the chromosomes gradually becoming long and irregular in form, and they spin out delicate threads which later become irregularly thickened. Gradually the chromatin material from the chromosomes becomes located in these threads. The outline of the chromosomes may still be recognized at this stage, but as the dispersion of the chromatin proceeds the boundaries of chromosomes can no longer be recognized. Secondary threads arise from the primary threads, and the chromatic material passes rapidly into them. Finally the nucleus becomes filled with a network of irregularly thickened threads, as shown in fig. 38. Later, one or more nucleoli appear together with chromatin granules of varying shapes and sizes.

Meiosis in *O. pratincola* mut. *formosa*

The nuclei of the pollen mother cells in mut. *formosa* during all the stages of meiosis and the following division resemble those in *O. pratincola* strain E.

Meiosis in *O. pratincola* strain C

The history of meiosis in strain C resembles that of strain E and mut. *formosa* in all respects. Careful observations were made on hundreds of nuclei of the pollen mother cells in this strain, in the hope of discovering some peculiarities distinguishing it from strain E and from mut. *formosa*, but nothing different was found. It may be stated confidently that if the slides of strain E, strain C, and mut. *formosa* were mixed it would be impossible to distinguish them.

Meiosis in *O. pratincola* strain M

It will be remembered that strain M is the cross mut. *formosa* pollinated by *f. typica* of strain C. It has the appearance of *O. pratincola* *f. typica*, the flat-leaved character of which dominates the revolute-leaved character of mut. *formosa*. The F_1 plants cannot be distinguished from strains E and C of *pratincola*, therefore, although they carry the revolute-leaved character of mut. *formosa* as recessive.

When self-pollinated, strain M gives an F_2 progeny showing simple monohybrid segregation of 3 typical *pratincola*: 1 mut. *formosa*. The strain was designated M by BLANCHARD (COBB 21) because of the Mendelian segregation.

All the stages in the course of meiosis in strain M show clearly that there are no fundamental differences between this form and the other strains discussed up to and including the second contraction. With the unfolding of the irregular threads of the second contraction, however, an entirely new situation is presented. All the strains discussed before show chains of fourteen chromosomes attached end to end in the later stages of prophase. Strain M has a closed chain of twelve chromosomes attached end to end, to which is linked a pair of chromosomes in the form of a ring (fig. 41 a). In the earlier periods of metaphase the ring of two chromosomes remains attached to the circle of twelve (figs. 42 a, 43 a), but during the late metaphase it breaks away and takes a position at one side of the spindle

(fig. 44 a). The chromosomes assume the form of thick V's and rods, due to condensation. The characteristic zigzag arrangement is clearly shown in the closed chain of twelve chromosomes, alternate chromosomes passing to the poles of the spindle. The chromosomes of the detached pair separate and accompany the two groups of six chromosomes as they pass to the poles. The chromosomes from the pair may be followed into late anaphase, but with the approach of the telophase each becomes associated with the other six chromosomes, and it is impossible to distinguish them. The pair of chromosomes is significant of the genetic behavior of strain M. These homologous chromosomes probably carry respectively the genes for flat leaf (*pratincola*) and revolute leaf (*formosa*), and their segregation and recombination in the breeding of strain M accounts for the simple Mendelian ratio peculiar to this monohybrid. The remaining stages of pollen formation in this plant are the same as those described in *Oenothera pratincola* strain E.

Formation of pollen grains

The four microspore nuclei grow rapidly and delicate fibers appear between them. These sometimes become prominent (fig. 45), resembling spindle fibers, but they soon disappear. Shortly small refractive vacuoles begin to develop in the cytoplasm (fig. 46). These are numerous at first, and distributed rather uniformly through the cytoplasm of the pollen mother cell. The regions of the mother cell between the nuclei infold at the periphery (fig. 46), nearly equidistant from the nuclei and at right angles to the former spindles; so that when the new walls are developed the resulting spores are tetrahedral in form. Later, vacuoles become arranged in the cytoplasm between the nuclei and enlarge, some of them fusing to form large flattened vacuoles (fig. 47). They finally become plates which extend from the center of the cell between the nuclei to the infolded regions at the periphery. The complete cleavage of the protoplasmic material appears to result from the furrows originating at the surface (fig. 48), which grow inward very rapidly, breaking through the large vacuoles. The furrows are thin at first but later become wider and conspicuous.

C. H. FARR (37, 38, 39, 40) has shown that the simultaneous divi-

sion of the pollen mother cells in *Nicotiana*, *Magnolia*, *Sisyrinchium*, and *Nelumbo* occurs by means of furrows. Before this time it was thought that the quadripartition was brought about in conjunction with cell plates formed between spindles. W. K. FARR (41) also reports a similar process in *Cobaea*, and CASTETTER (12, 13) for *Melilotus* and *Cucurbita*. CLELAND (15, 16, 18, 19) has noted an invagination process, which he does not fully describe, in *Oenothera franciscana*, *O. biennis*, *O. biennis sulfurea*, and "*O. muricata*." The present studies on *Oenothera pratincola* agree with his observations so far as he records them. He does not refer to the vacuolation which precedes the final wall formation.

A membrane appears very soon around each young microspore, first recognized as a delicate film lining each cell cavity of the tetrad. It is distinct from the wall of the pollen mother cell although in most intimate contact with it; this membrane presently thickens. The young pollen grain is bluntly tetrahedral, with the peripheral side infolded to form a basin. The cell cavity is densely filled with protoplasm and there are no large vacuoles. The concavity of the basin is directed toward the apex of the tetrahedron. The wall is extremely thin at the three peripheral angles of the pollen grains. Shortly it thickens considerably and a mucilaginous material develops at these three angles. The little disks of mucilage enlarge and extend somewhat laterally. FRITSCHE introduced the name "Zwischenkörper" for these mucilaginous disks, and the same name has been used by NÄGELI and STRASBURGER. BEER (7) calls them interstitial bodies. The normal pollen grains of *O. pratincola* have three of these interstitial bodies. Some have only two and still others have only one. These cases are probably abnormal. The pollen grains of *gigas* forms of *Oenothera* have four or more of these disks. Very soon the wall of the pollen mother cell breaks down and the pollen grains are set free. As the pollen grain enlarges, the three interstitial bodies become more and more prominent, finally becoming three lobes. This gives the peripheral face of the pollen grain a more pronounced triangular appearance. Shortly a secondary thickening develops within the first pollen wall, extending over the whole of the inner face of the pollen membrane but very thin at the three lobes. A disk-like partition is formed across the base of each lobe. This disk con-

sists of two parts, the outer part a dense homogeneous layer and the inner part a less dense stratified lamella which is cap-shaped. The cavities of the three lobes at this stage no longer contain the mucilaginous substance which earlier takes a brilliant stain with haematoxylin. BEER (7) suggests that this mucilaginous substance is used in the formation of the closing disks. He thinks that the closing disk is later eaten away by a solvent, probably an enzyme secreted by the protoplast. The thickening of the pollen wall continues in the regions between the lobes. My observations on the development of pollen in *O. pratincola* are in accord with those of BEER for *O. biennis* and *O. longiflora*.

Cytological discussion

The conclusions of GATES (42, 43, 46), DAVIS (22, 23, 24), GEERTS (48), CLELAND (15, 16, 18, 19), VALCANOVER (88), and HÅKANSSON (53) are all in substantial agreement that the arrangement of the chromosomes in *Oenothera* is telosynaptic. Only one investigator, BOEDIJN (9, 10), maintains it to be parasympatic, and his observations are at variance with those of the investigators just cited. BOEDIJN reports for *Oenothera lamarckiana*, during the early part of the heterotypic prophase, the side-by-side conjugation of the thick threads. From this stage he passes to the stage known as the second contraction, and figures pairs of long slender threads (two chromosomes) twisted in such a manner as to give the appearance of normal strepsinema stage. In early diakinesis stages he observed seven pairs of chromosomes, often entirely separate from one another and sometimes forming rings, and states that the single chromosomes comprising these pairs may split partially during this stage. SINOTÓ (81) reports the pairing of homologous chromosomes in *O. lamarckiana*. He does not think that they are parasympatically arranged (82) after the beginning of the prophase in *O. sinuata* L. CLELAND (17) finds in *O. lamarckiana* at diakinesis a circle of twelve chromosomes and a ring of two, in contrast to the seven pairs described by BOEDIJN at this stage. GATES (42), GEERTS (48), DAVIS (24), and HÅKANSSON (53), who have also worked on the cytology of *O. lamarckiana*, do not report any evidence that would lead one to believe in the parasympatic interpretation proposed by BOEDIJN.

It is worth noting here that some genera related to *Oenothera* have been stated to show parasympatic arrangement of the chromosomes. TÄCKHOLM (86) for *Lopezia*, MICHAELIS (64) and SCHWEMMLE (72) for *Epilobium*, HÄKANSSON (52) for *Godelia*, and SCHWEMMLE (73) for *Eucharidium* report parasympasis. SCHWEMMLE (72) reported telosympasis in *Oenothera*, but changed his interpretation through conclusions reached in a cytological study of *Eucharidium concinnum* (73). He describes for *Eucharidium* three methods of gemini formation, one of which (Reihe C) resembles that of *Oenothera* in some stages. His figures, apparently of second contraction (figs. 27-29, Taf. V), resemble those of CLELAND for this stage (15 figs. 15, 21; 16 fig. 5). The interpretations put on apparently similar figures by the two investigators, however, are different. CLELAND and others believe that the chromosomes that emerge from second contraction result from the segmentation of a univalent spireme. SCHWEMMLE, on the contrary, maintains that during the second contraction the chromosomes are already in pairs. He gives some figures (figs. 2-5, 12-14, 20-22) of the stages prior to second contraction. These stages are critical for the determination of telosympasis or parasympasis in any species. None of these figures resembles the stages that I have observed in *Oenothera pratincola*, nor do they resemble the figures of other investigators of *Oenothera* cytology, with the possible exception of certain of BOEDIJN'S (10) figures of the early prophase. The reported absence of these stages in *Oenothera*, which according to SCHWEMMLE determine telosympasis or parasympasis, is believed by him to be due to environmental conditions, such as temperature and other undecided factors, which suppress their appearance. He suggests that as a result of these conditions the normal development of the anther and the pollen mother cell is in some cases retarded and in other cases prolonged. He predicts that it may become possible to demonstrate parasympasis in *Oenothera*. It must be remembered that *Eucharidium* and *Oenothera* are distinct genera, and that conclusions for *Oenothera* through a study of *Eucharidium* may not hold good for the former.

In a recent paper, SCHWEMMLE (74) reports on the cytology of the cross *Oenothera berteriana* \times *Oenothera (Onagra) "muricata"*. Both parents have fourteen chromosomes, but those of the latter are twice

as large as those of *berteriana* (as observed during the early anaphase). All the stages of meiosis in this hybrid, from the heterotypic prophase to the untangling of the second contraction, are similar to the stages usually met in the meiosis of species of *Oenothera*. At diakinesis the chromosomes of "*O. muricata*" form a closed chain, as shown by CLELAND (19); the arrangement of the chromosomes in *O. berteriana* at this stage is not known. SCHWEMMLE states that the hybrid shows great variation in the arrangement of the chromosomes at diakinesis. He reports in some nuclei two small chains of three chromosomes arranged end to end, a pair of chromosomes, and six single chromosomes; in other nuclei many pairs; and in still others chains of chromosomes of large number accompanied by fewer univalent chromosomes. Cases of small chromosomes pairing with large ones are also reported by SCHWEMMLE. When the nuclear membrane dissolves, prior to metaphase of the heterotypic mitosis, six of the larger chromosomes of "*O. muricata*" may be recognized; but the seventh is not to be clearly seen (74). The small chromosomes of *O. berteriana* are likewise seen at this stage. The arrangement of the paternal and maternal chromosomes on the spindle at the heterotypic metaphase is variable. During the late metaphase, when some of the chromosomes have nearly reached the pole, others are seen lagging in the mid-region of the spindle. The distribution of the chromosomes to the two poles during anaphase is also variable. SCHWEMMLE observes the following cases: (1) four large three small to one pole and three large four small to the other; (2) five large two small to one pole and two large five small to the other; (3) six large one small to one pole and one large six small to the other; (4) seven large to one pole and seven small to the other. He finds abundant spindles with unequal chromosome numbers at anaphase, resulting in irregular distribution of the chromosomes to the two poles. He suggests that this irregularity is due to the fluctuations of the autumnal temperature when the material was fixed. He concludes that in a cross between two species of *Oenothera* presenting size differences in the chromosomes, the paternal and the maternal can be recognized and their distribution during meiosis observed.

The four strains under discussion, *O. pratincola* f. *typica* strain E, mut. *formosa*, f. *typica* strain C, and strain M, have been care-

fully examined and the arrangement of the chromosomes in all of them was found to be telosynaptic. All these strains will be treated together in this discussion because they show the same characteristic behavior. During the stages of the early heterotypic prophase there is hardly any evidence suggestive of parasynapsis. When the threads thicken and contract some of them may be found side by side, but such chance parallelism is to be expected. By careful and detailed observations it becomes evident that the formation of the spireme in the heterotypic prophase is effected by an irregular process of condensation, rather than by a side-by-side approximation of two distinct thread systems. During the open spireme stage following synizesis the threads are uniformly single, and there is no evidence of a bivalent spireme. During the second contraction following the open spireme stage, when the chromatin threads are thrown into radiating loops, these loops are also univalent. The most significant evidence that the chromosome arrangement in these strains is telosynaptic is presented with the unfolding of the chromatin threads at the end of the second contraction, from which emerges a closed chain of chromosomes attached end to end. There is no doubt that this chain is the outcome of the segmentation of the spireme at the termination of the second contraction stage.

The arrangement of the chromosomes in closed chains at the end of the second contraction stage, together with the extreme regularity with which all the stages are executed, leads CLELAND to conclude that the delicate threads of the resting nucleus in the species of *Oenothera* which he has investigated give rise to chromosomes definitely placed end to end and not to chance arrangements.

In most plants and animals the homologous chromosomes at the stage termed diakinesis are distributed in pairs throughout the nucleus. In *Oenothera* the situation at this stage, with the exception of a few species, is different. In most of the species of *Oenothera* which have been cytologically studied, a closed chain of some or of all of the chromosomes is found. Various types of chain formation have been described. In *O. franciscana* CLELAND (15) reports a closed chain of four chromosomes and five pairs; the chain of four, however, breaks into two pairs of chromosomes at the metaphase; in *O. biennis* and *O. biennis sulfurea* CLELAND (18) and EMERSON (32) ob-

served no pairing at all but two chains, one of eight chromosomes and the other of six, an arrangement which VALCANOVER (88) and KIHARA (54) confirm for *O. biennis*. In both *O. franciscana sulfurea* and *O. lamarckiana* CLELAND (16, 17) found a chain of twelve chromosomes to which is attached a ring of two chromosomes. His finding is confirmed by HÅKANSSON (53) for *O. lamarckiana*. Finally, SCHWEMMLE (72) in *O. rosea* and CLELAND (19) in "*O. muricata*" discovered a closed chain of all the fourteen chromosomes. Only in *O. blandina* and *O. deserens* has CLELAND (17) found seven independent pairs of chromosomes. SCHWEMMLE (72) also found seven independent pairs of chromosomes in *O. hookeri*.

When chains of chromosomes are present it becomes a matter of great interest to determine how the distribution of their chromosomes to the two poles takes place. CLELAND holds that the zigzag arrangement of the chromosomes at the heterotypic metaphase explains the situation. He states that in all the species of *Oenothera* he has investigated, where there are chains, the alternate chromosomes of the chains, because of their fiber attachments, generally pass to the same pole during the heterotypic metaphase, thus effecting the segregation of the homologous chromosomes. VALCANOVER (88), HÅKANSSON (53), and KIHARA (54) observed the characteristic zigzag arrangement described by CLELAND at this stage. EMERSON (32) did not see the zigzag arrangement in *biennis* as described by CLELAND, and consequently did not believe that the alternate chromosomes go to the same pole. He reported that the separation of the chromosomes is irregular. He suggested that *O. biennis* has fourteen different chromosomes instead of seven pairs of homologous chromosomes, and the chromosomes of this species are not homologous at all because they do not pair.

In all the strains of *O. pratincola* and *mut. formosa* the chromosomes are distributed to the opposite poles in the characteristic zigzag manner described by CLELAND, with occasional irregularity however. Whether such a zigzag arrangement leads to the separation of the homologous chromosomes, as assumed by CLELAND, and whether the chromosomes in *Oenothera pratincola* are homologous cannot definitely be stated for the present. In light of the genetical behavior of these strains, the maternal and paternal sets of chromo-

omes come out of meiosis as they entered the zygote. The only exceptions are due to irregularities in the zigzag arrangement. It appears from the genetic behavior, to be discussed in the following section, that all the chromosomes in *O. pratincola* are not homologous.

As discussed, a closed chain of fourteen chromosomes is formed in the final prophase stages, both in *f. typica*, strain C, and *mut. formosa*. Following a cross between *mut. formosa* (revolute leaves) and strain C (flat leaves), the F_2 homozygous dominant (flat-leaved) shows at this stage not a closed chain of fourteen chromosomes, but a circle of twelve chromosomes and a ring of two chromosomes attached to it. The question that presents itself is why and how the closed chain of fourteen chromosomes breaks into a closed chain of twelve chromosomes and a ring of two. An interpretation offered by Dr. F. COBB BLANCHARD is given in the genetical discussion to follow.

The nature and function of the nucleolus have been the subject of much discussion in older as well as in more recent cytological literature. Some investigators believe that the nucleolus is merely a secretion product of the nucleus. A great number of the older writers (for literature see WAGER 93), as well as modern cytologists, such as LENOIR (58, 59), LITARDIÈRE (60), TAMURA (87), VAN CAMP (89), and many others believe, however, in the transportation theory. In *Oenothera*, CLELAND (15, 16, 18, 19) and BOEDIJN (9) support the transportation theory. SINOTÔ (82), working with *O. sinuata* L., finds no evidence for the transportation theory; that is, finds no evidence that the nucleolus acts as a storage structure.

The nucleus of pollen mother cells of *O. pratincola* contains at least one large spherical nucleolus. Sometimes more than one may be present. During the late synizesis some of the threads connected with the nucleolus become greatly enlarged, as though material had suddenly been transferred to them. This suggests that the nucleolus acts as a reservoir, from which chromatic substance is drawn to build up the spireme in the final stages of the heterotypic prophase. The nucleolus in the resting nucleus stains very deeply with haematoxylin, but very little of the stain is held in later stages of prophase, probably because the stainable material is carried away to build up

other structures. When the nucleolus is divested of all of its coloring matter, it shows in its interior a very small spherical body, the endonucleolus. This minute structure is sometimes connected with the spireme, but in most cases lies freely in the interior of the nucleolus. It disappears during the late metaphase. During interkinesis many nucleoli develop in contact with the chromosomes, arising always *de novo*; hence it appears highly probable that the nucleolus is a storage organ for material, akin to chromatin, which is formed anew in each nucleus.

The segmentation of protoplasm by cleavage furrows was observed by BÜTSCHLI (11). He interpreted the process as the result of the higher surface tension at the equator of the cell caused by the flow of the protoplasmic currents toward the centrosome. McCLENDON (61, 62, 63), SPECK (83, 84), and CHAMBERS (14) corroborate BÜTSCHLI's theory by their own observations, and present strong evidence in support of it. KITE (55), however, maintains that cleavage is due to "concomitant shrinkage and swelling or change in water-holding power of the different portions of the cytoplasm." GRAY (49) thinks that the cleavage furrow is due to an equilibrium established between the effect of the movement in the protoplasm and the surface tension on the surface of the cell. CASTETTER (12) agrees with BÜTSCHLI's theory and its supporters.

The division of the protoplasm to form the pollen grains in *O. pratincola* is a process of segmentation through cleavage furrows. Careful observation shows that the granular cytoplasm moves toward the nuclei from the regions of the protoplasm equidistant from the nuclei. This results in vacuolate hyaline regions between the nuclei. The smaller vacuoles fuse to form larger ones arranged between the nuclei. The cleavage furrows progress from the periphery very rapidly, meeting the vacuoles with which they fuse. The process of cleavage is greatly assisted by the presence of the vacuoles with which the furrows fuse as they progress inward.

Genetical discussion

In many species of *Oenothera* chromosomes do not pair at diakinesis; such is the case in *Oenothera pratincola*. The failure of chromosomes to pair in this genus is presumably due to lack of the

usual affinity resulting from differences in their genetic constitutions. Many explanations have been offered to account for this unusual phenomenon. According to CLELAND (18), chromosomes which pair in diakinesis are relatively homozygous and those which do not are relatively heterozygous. CLELAND (15, 16, 18) suggests that the chain formation is probably due to hybridity. HÅKANSSON (52), in his paper on *Godetia*, states that hybrids present at diakinesis a strong tendency to form circles. On the other hand, GATES (47) finds a regular pairing of the chromosomes in a hybrid of *Oenothera*. OEHLKERS (68) finds in *Oenothera* that there are hybrids with regular pairings as well as those with chains; hence the hypothesis that the formation of rings in certain species of *Oenothera* is an indication of their hybridity, seems untenable. CLELAND (18) also suggests two other ways in which the incompatibility of the chromosomes might have arisen. The first is that it may be due to gradual accumulation of gene mutations within a chromosome. This process, he thinks, is probably aided by the appearance of balanced lethal factors. The second is that, when the chromosomes are arranged end to end, it may be more difficult for them to pair than it is when the homologous chromosomes are placed side by side.

In explanation of the genetical phenomena which *Oenothera pratincola* exhibits, a hypothesis was formulated by BARTLETT (6) which assumes that two unlike types of gametes are produced, which he calls α and β . Generally the functional egg is an α gamete, and the functional sperm a β gamete, the β eggs and α sperms usually failing to function. When a zygote is formed in *O. pratincola*, therefore, it generally has the constitution $\alpha\beta$. It receives the α determiners of the female parent and the β determiners of the male parent. The results of intercrosses between the entire group of forms indicate that the differences in the characteristics of the several types are in general determined by the α gamete, and thus the modifications of the α gamete are responsible for the greater number of mutations in *Oenothera pratincola*. This is shown by the matroclinal inheritance which the mutations under discussion display in crosses with their parent form. In general, pollination by the β gamete of a mutation has the same effect as by a β gamete of *f. typica* from which the mutation arose. There are very few characters

that are affected by different sources of the β gamete. Among the mutations that have been genetically studied, probably there is no mutation of $\alpha\beta$ constitution effected by a change in the β gamete alone except the one reported by DE VRIES (91) in the case of *O. biennis sulfurea*. This mutation shows patroclinic inheritance in crosses with its parent.

According to BARTLETT's hypothesis, the characteristic portion of the α and the β gametes may consist of a single chromosome or a group of chromosomes. The chromosomes of the α and the β groups do not segregate according to the law of chance in reduction division; instead they pass together as groups to the opposite poles. They are passed to the daughter nuclei as distinct sets, one from the maternal and the other from the paternal parent. In addition to the characteristic chromosome or chromosomes, there may be in some forms chromosomes which are freely segregating. These carry factors for characteristics which show Mendelian inheritance.

For the better understanding of the discussion that is to follow, it is rather important to understand thoroughly what is meant by homologous chromosomes. In most plants and animals, and some of the species of *Oenothera*, when the chromosomes pair during diakinesis, and probably carry allelomorphic factors, such chromosomes are referred to as homologous. In cases when the chromosomes do not pair, and are not known to carry allelomorphic factors, the use of the term homologous chromosomes is rather confusing, because of our present day conception of the term in connection with Mendelism. Some of the chromosomes of *O. pratincola* are not homologous.

When different strains of *Oenothera pratincola* are intercrossed they show a behavior which is very significant genetically. Mut. *formosa* (revolute leaves) is given by strain E (flat leaves). In crosses between the two forms the inheritance is matroclinic, as shown by BARTLETT (6) and COBB and BARTLETT (20). This behavior of mut. *formosa* shows that it arose from strain E through a change in the α gamete of E. When f. *typica* of strain C is pollinated by mut. *formosa*, the F₁ progeny consists of plants in appearance exactly like f. *typica*. All the F₂ plants resemble f. *typica*. If mut. *formosa* is used as the pistillate parent and f. *typica* of strain C as the staminate

parent, all the F_1 individuals are flat-leaved, resembling strain C. When these F_1 individuals are self-pollinated, they give in the F_2 generation a ratio of three flat-leaved plants to one having revolute leaves. This simple Mendelian segregation continues in the following generations, as shown by COBB (21). The flat-leaved hybrid strains from this cross (mut. *formosa* \times f. *typica* strain C) have been designated as *O. pratincola* strain M by BLANCHARD (COBB 21), and one homozygous flat-leaved strain has been carried under that name for the last seven years. A closed chain of fourteen chromosomes is formed in the final prophase stages (diakinesis), both in mut. *formosa* and in strain C. The homozygous dominant strain M, however, shows at this stage a closed chain of twelve chromosomes to which is attached a pair of chromosomes. These two chromosomes are homologous and contain the factors for flat and for revolute leaves. Such a pair of chromosomes should be expected, in view of the fact that the cross mut. *formosa* \times f. *typica* strain C mendelizes and gives a monohybrid ratio in the F_2 generation.

As has already been discussed, certain irregularities occur during the heterotypic metaphase, through the deviation of the chromosomes from the characteristic zigzag arrangement, which sometimes result in the abnormal distribution of the chromosomes to the two poles, eight chromosomes going to one pole and six to the other instead of seven and seven. In certain cases, even though the number of chromosomes distributed to each of the two poles is normal, the paternal and the maternal set exchange a chromosome or chromosomes. This gives an abnormal result in breeding. The pollen grain with eight chromosomes may possibly function and give rise to fifteen-chromosomed individuals. The pollen grains with six chromosomes probably do not function at all, as no species have been discovered so far with only thirteen chromosomes. These two cases will be omitted entirely from the discussion that is to follow. The complexes that can arise through an irregularity, even though the distribution of the number of chromosomes to the two daughter nuclei is normal, are many and varied, depending on the nature of the irregularity, the point at which it occurred, and its relation to individual chromosomes, etc. These irregularities have been thoroughly discussed by CLELAND (18, 19). He has also calculated the

theoretical expectancies and the genetical results arising therefrom in case of some such complexes.

From the results of her breeding experiments, FRIEDA COBB BLANCHARD has formed a hypothesis of the constitutions of *O. pratincola* and its revolute-leaved mutations. Her paper dealing with this problem will probably precede the present paper in publication. The hypotheses which she offers have been made known to the writer, and he has adopted them in explanation of his cytological findings. Her explanation of the origin of *mut. formosa* is given in the following paragraph.

Let 1, 2, 3, 4, 5, 6, 7 represent the chromosomes of the α gamete of *O. pratincola* strain E and 8, 9, 10, 11, 12, 13, 14 represent the chromosomes of the β gamete. Then the zygote formed will have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 as its chromosome constitution. This zygote in reduction division will in turn produce α gametes having chromosomes 1, 2, 3, 4, 5, 6, 7 and β gametes having 8, 9, 10, 11, 12, 13, 14. Now suppose an irregularity in the reduction division to occur in the production of the eggs, and chromosome 7 of the α gamete to dissociate from the other members of its series and to become interchanged with chromosome 14 of the β series, so that chromosome 7 goes to the β complex and chromosome 14 to the α complex. In such a case we shall have 1, 2, 3, 4, 5, 6, 14 for the α gamete and 8, 9, 10, 11, 12, 13, 7 for the β gamete. (For an explanation of the conception of this type of a mutation see the section entitled "Whole-chromosome crossovers" in BLANCHARD and BARTLETT 8.) If this modified α egg is fertilized by a regular sperm of *O. pratincola* strain E having 8, 9, 10, 11, 12, 13, 14 as its chromosome constitution, the zygote will have the constitution 1, 2, 3, 4, 5, 6, 14, 8, 9, 10, 11, 12, 13, 14. There will be a pair of identical chromosomes in this combination. BLANCHARD believes that *mut. formosa* arises by such a whole chromosome cross-over, the chromosome which becomes duplicated being that one of the β complex of strain E which carries the recessive factor for revolute leaves. If this assumption is correct, *mut. formosa* might be expected to show a pairing of two of the chromosomes during the final prophase stages (diakinesis). Apparently, however, there is no such free pair, but it is conceivable that a pair of homologous chromosomes might not

be able to break away from the closed chain. Several others of the revolute-leaved mutations of strain E are probably the results of whole chromosome crossing-over of the type just discussed, where one or more chromosomes from one complex exchange their places with chromosomes of the other complex.

Linkage in *Oenothera* has been the subject of some discussion in recent literature. SHULL (78, 79) thinks that, except for the *brevistylis* and old gold (80) characters, all the known factors of *Oenothera* probably lie in a single chromosome pair. In this pair SHULL believes that he has located nine factors, affecting visible characters, with which two gametic and two zygotic lethals are supposed to be associated. EMERSON (31) has shown that the data offered by SHULL in support of this hypothesis have some discrepancies, and that they do not establish the correctness of the hypothesis.

It appears that linkage in *Oenothera* is due to the cohesion of the chromosomes, and their separation in the heterotypic division in such a way that the maternal set goes to one pole and the paternal set to the other pole of the spindle. It is probable that in *O. pratincola* the maternal and paternal members of the chromosome set alternate with each other, and the characteristic zigzag arrangement at the heterotypic metaphase effects their separation; thus the paternal and maternal sets of chromosomes come out as they enter the zygote. Hence in *O. pratincola* the characteristic group of α chromosomes behaves as a single chromosome and is responsible for a group of linked characters. Interchange of a whole chromosome or chromosomes between the α and the β complex does occur as a result of an irregularity in the zigzag arrangement of the chromosomes during the heterotypic metaphase. When such an interchange of chromosomes takes place (a "whole chromosome cross-over") it probably gives rise to a mutation. One case of this type has already been discussed.

GATES (46) holds that partial linkage and crossing over probably cannot occur in *Oenothera* because of the telosynaptic arrangement of the chromosomes. Due to such an arrangement, the chromosomes cannot twist about one another during the strepsenema stage and exchange chromatin material, as they do in most plants; hence "crossing over" may not always be possible. CLELAND (18) observes

the appressed sides in one or more peripheral loops during late stages of second contraction. These, he thinks, have apparently become twisted about each other in a way that might conceivably result in the exchange of chromatin particles. He thinks crossing over of this type is possible. HÅKANSSON (53) also thinks that there is a possibility of crossing over occurring in the second contraction. SHULL (80) maintains that the genetical evidence of linkage and crossing over is unequivocal in his cultures. He observes new and striking examples of it every season. *O. pratincola*, which has been under cultivation and has furnished material for the genetical researches of BARTLETT and BLANCHARD for the last fifteen years, does not as yet show any crossing over which cannot be explained by whole chromosome crossing over.

Summary

1. The diploid chromosome number in *Oenothera pratincola* is fourteen. The chromosomes at metaphase of the somatic mitosis are rod-shaped, variously bent, and are formed by the cross segmentation of the spireme.
2. The resting nuclei of the pollen mother cells are characterized by a network of delicate threads. Chromatin granules varying in size are scattered through the chromatic network, but more thickly at the periphery.
3. There is one large nucleolus and some smaller nucleoli. All of these bodies are in intimate contact with the chromatic network, and probably contribute their material to the spireme developed during the heterotypic prophase. An endonucleolus is present and may be observed from synizesis on to the heterotypic metaphase. The nucleolus is believed to be a storage organ for material related to chromatin, which is drawn upon in the development of the spireme during the heterotypic mitosis.
4. The approach of the heterotypic prophase is indicated by occasional parallel arrangements of the chromatic threads, but these are not very conspicuous. A gradual thickening of the threads is brought about by condensation and the movement of chromatic material along the threads. There is no indication of the fusion of two parallel threads at this stage.

5. As the nucleus enters the prophase, the threads attached to the nuclear membrane seem to contract, probably through condensation, and become gathered to form a ball of threads, the synizetic knot.

6. During synizesis many threads disappear, having delivered their chromatin contents to the threads which are to survive. These become prominent and uniform, and a spireme arises as the knot gradually expands. Some parallelisms are also to be noticed during synizesis, but these are certainly due to the irregular contraction and condensation that take place. Conditions indicate that there is no association or fusion of the separated halves of a univalent spireme or of two univalent spiremes to form a bivalent spireme.

7. The open spireme stage results from the gradual expansion of the synizetic knot, and presents threads of almost uniform thickness throughout the spireme. No free ends were observed in the thread system.

8. By the further contraction and condensation of the threads of the open spireme, the stage known as the second contraction is reached. During this stage all the chromatic material is gathered at the center of the nucleus, the threads in the center thickening more rapidly. Loops of the threads are not lost, even during the stage of greatest contraction, but they become much smaller and radiate from the central mass of chromatic material. These loops are uniformly single threads.

9. The loops could be traced throughout all stages of second contraction, and undoubtedly give rise in part to the univalent chromosomes which emerge at the end of the second contraction. The univalent chromosomes are arranged end to end, that is, telosynaptically.

10. At the stage known as diakinesis in most plants and animals, a closed chain of fourteen chromosomes attached end to end is found in *O. pratincola* strain E, which convincingly points to a telosynaptic association. Occasionally the closed chain breaks at some point to form an open chain of fourteen chromosomes.

11. The chain of chromosomes passes to the equatorial region of the spindle, where the spindle fibers become attached in such a way that alternate chromosomes pass to the same pole. There is a characteristic zigzag arrangement of the chromosomes at metaphase.

12. Anaphase is mostly normal, seven chromosomes passing to each pole; but there are about 3 to 7 per cent of exceptions, due to irregularities in the zigzag arrangement of the chromosomes which result in six chromosomes passing to one pole and eight to the other.

13. A lengthwise split of the chromosomes in preparation for the homeotypic mitosis is effected in late telophase of the heterotypic division.

14. During interkinesis the halves of the split chromosomes (daughter chromosomes) are connected in their middle region; but the ends are widely separated so that each pair resembles an X.

15. The homeotypic divisions are regular and occur simultaneously. The two spindles lie sometimes in the same plane and sometimes at right angles to each other.

16. All of the stages of meiosis in the pollen mother cells of *O. pratincola* strain C are identical with those of *O. pratincola* strain E and of its mut. *formosa*.

17. Strain M (*formosa* \times strain C) agrees with *O. pratincola* strain E, strain C, and mut. *formosa* in all the stages of meiosis, up to and including second contraction. Following second contraction, in strain M there emerges a closed chain of twelve chromosomes, with a ring of two attached to it. The ring of two chromosomes breaks away from the closed chain of twelve at the heterotypic metaphase, and the two chromosomes of the pair pass to different poles.

18. This pair of chromosomes is significant of the genetic behavior of strain M. The chromosomes are probably homologous and carry the genes for flat leaf and revolute leaf, and their segregation and recombination in the breeding of strain M give the simple Mendelian ratio.

19. The grand-daughter nuclei pass into a resting condition in which the outlines of the chromosomes become lost in the chromatic network. The process is one of expansion and irregular distribution of the chromatin throughout the nucleus.

20. The segmentation of the protoplasm to form the four pollen grains is effected by furrows which start at the periphery of the pollen mother cell (between the four nuclei) and grow inward, cutting through regions of vacuoles as they pass to the center of the cell.

21. The position of the chromosomes in the chain is believed to be fixed, the maternal alternating with the paternal.

22. The failure of the chromosomes to pair in the late prophase of the heterotypic mitosis is probably because of unlike genetical constitution, but the hypothesis that the formation of chains in certain species of *Oenothera* is an indication of their hybridity is believed to be untenable.

23. *O. pratincola* produces two types of gametes, called α and β . Generally the functional egg is an α gamete and a functional sperm a β gamete.

24. Irregularities in chromosome distribution during the heterotypic metaphase result at times in an exchange of chromosomes, so that an α chromosome enters a β complex and vice versa, and such interchange of chromosomes is probably the origin of certain mutations. By the theory of FRIEDA COBB BLANCHARD, presented in the genetical discussion, the origin of *mut. formosa* from *O. pratincola* strain E is due to such an interchange of chromosomes.

25. Certain examples of linkage in *Oenothera* are probably due to cohesion of non-homologous chromosomes in groups. Crossing-over has not been observed in *Oenothera pratincola*.

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UNIVERSITY OF MICHIGAN
ANN ARBOR, MICH.

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EXPLANATION OF PLATES VII-IX

All figures were sketched with the aid of a camera lucida, under a Spencer microscope, with Spencer 1.8 oil-immersion objective and 18 \times eye-piece; they have been reduced here one-fourth in reproduction; present magnification approximately 1400 diameters. *Oenothera pratincola* strain E unless otherwise stated.

PLATE VII

FIG. 1.—Metaphase of mitosis in archesporial cell viewed from pole of spindle, showing fourteen chromosomes.

FIG. 2.—Early anaphase of mitosis in archesporial cell.

FIG. 3.—Resting nucleus of pollen mother cell.

FIG. 4.—Gradual thickening of threads, with occasional parallelism, approaching prophase of heterotypic mitosis.

FIG. 5.—Advanced stage in thickening of threads, giving a coarse reticulum.

FIG. 6.—Beginning of contraction of reticulum prior to synizesis; threads varying greatly in thickness.

FIG. 7.—Early synizesis showing irregular thickening of threads.

FIG. 8.—Mid-synizesis.

FIG. 9.—Loosening of synizetic knot; threads somewhat thicker and more uniform.

FIG. 10.—Open spireme emerging from synizesis; note mass of contracted threads at left.

FIG. 11.—Open spireme, single threads.

FIG. 12.—Open spireme, thread showing chromomeres.

FIG. 13.—Second contraction; note thickening of threads in center and peripheral loops.

FIG. 14.—Second contraction, showing thick threads just before differentiation of chromosomes.

FIG. 15.—Second contraction; three chromosomes distinct.

FIG. 16.—Second contraction; chromosomes evident.

PLATE VIII

FIG. 17.—End of second contraction stage; chromosomes distinct but still crowded.

FIGS. 18-20.—Closed chains of fourteen chromosomes in late prophase.

FIG. 21.—Open chain of fourteen chromosomes in late prophase due to break in one of attachments.

FIG. 22.—Closed chain of fourteen chromosomes; multipolar spindle omitted.

FIG. 23.—Open chain of fourteen chromosomes; multipolar spindle.

FIG. 24.—Heterotypic metaphase showing regular zigzag arrangement of chromosomes; alternate chromosomes go to same pole.

FIG. 25.—Two chromosomes (*a*, *b*) in different parts of chain suspended between the poles.

FIG. 26.—Two pairs of adjacent chromosomes (*a*, *b*) passing to different poles of spindle.

FIG. 27.—Pair of adjacent chromosomes (*a*) passing to one pole with single chromosome (*b*) suspended on equatorial plate.

FIGS. 28-30.—Chromosome groups with fiber attachments so irregular that distribution of chromosomes is uncertain.

FIG. 31.—Late anaphase, polar views.

FIG. 32.—Late anaphase, showing unequal distribution of chromosomes (6 and 8).

PLATE IX

FIG. 33.—Late anaphase, polar view, showing typical arrangement of chromosomes.

FIG. 34.—Interkinesis, showing split chromosomes and formation of new nucleoli.

FIG. 35.—Interkinesis, showing X-like pairs of daughter chromosomes with denser regions.

FIG. 36.—Homeotypic prophase with pairs of daughter chromosomes now condensed; an example of irregular distribution, six chromosomes in one nucleus and eight in other; note prominent split in chromosomes.

FIG. 37.—Homeotypic metaphase; side view of one plate and polar view of other, showing regular distribution of chromosomes.

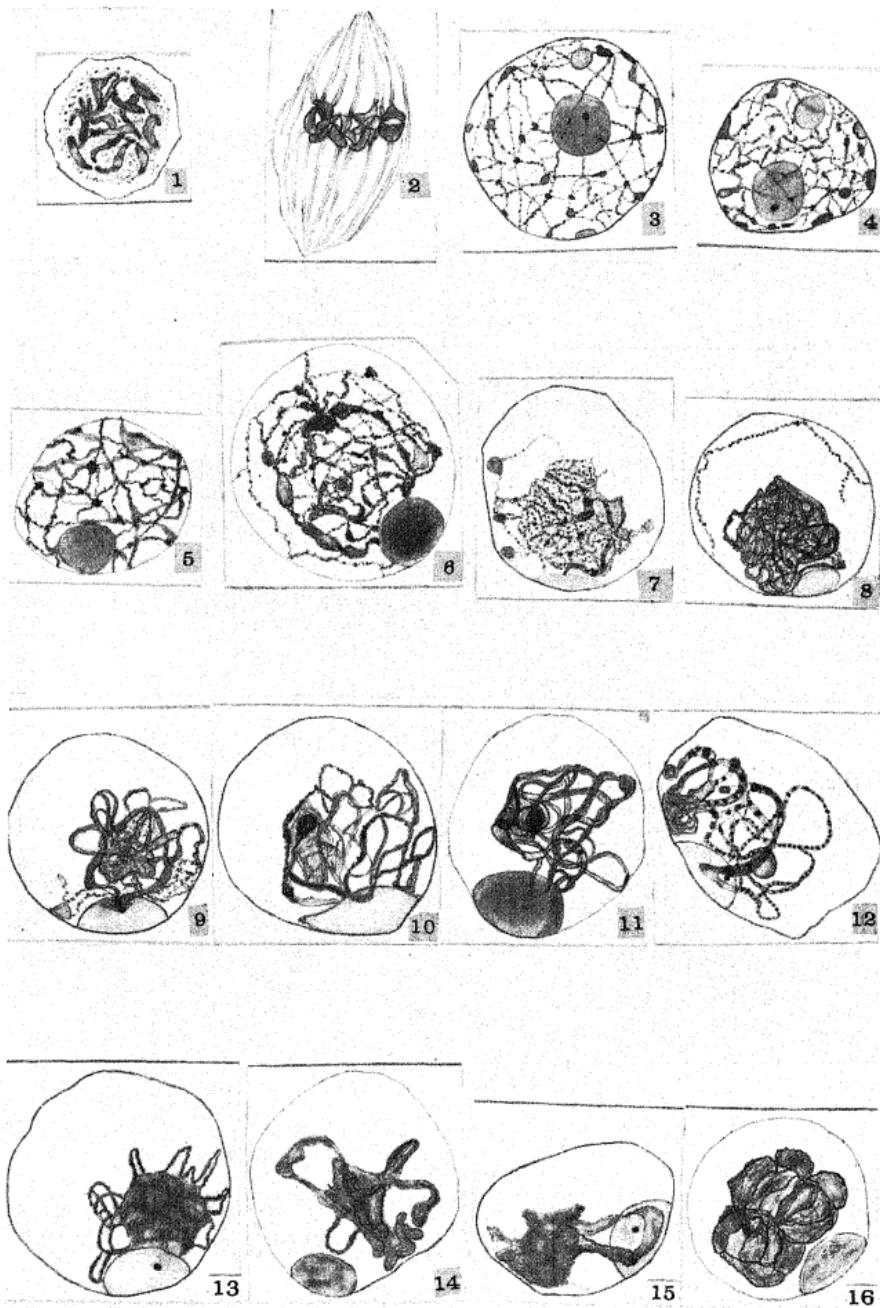
FIG. 38.—Telophase of homeotypic mitosis, chromosomes spun out into threads forming irregular network.

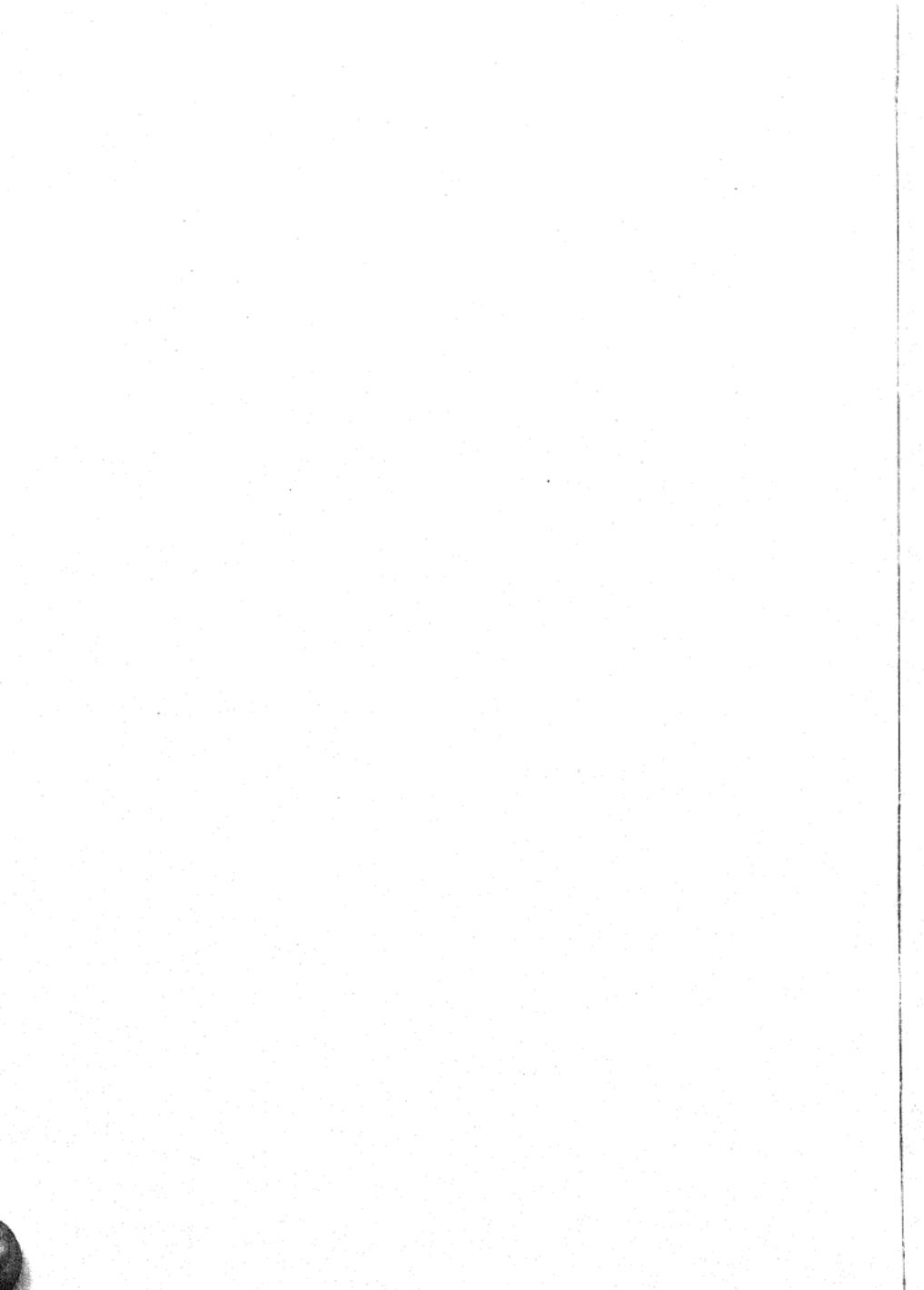
FIG. 39.—Closed chain of fourteen chromosomes in mut. *formosa*; late prophase.

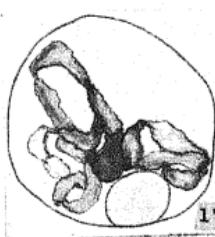
FIG. 40.—Open chain of fourteen chromosomes in mut. *formosa*, result of break in chain; late prophase.

FIG. 41.—Closed chain of twelve chromosomes and ring of two (*a*) in strain M; late prophase.

FIG. 42.—Closed chain of twelve chromosomes and ring of two (*a*) in strain M; multipolar spindle omitted.



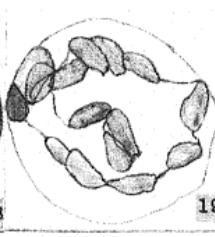




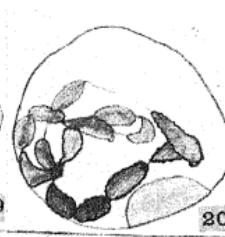
17



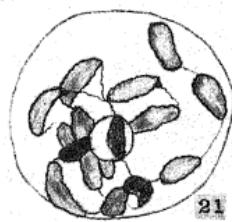
18



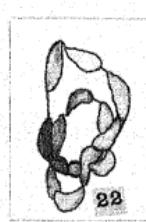
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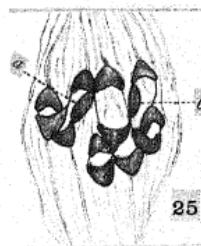
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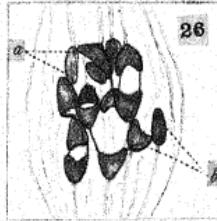
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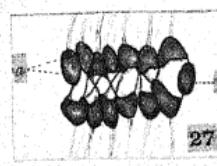
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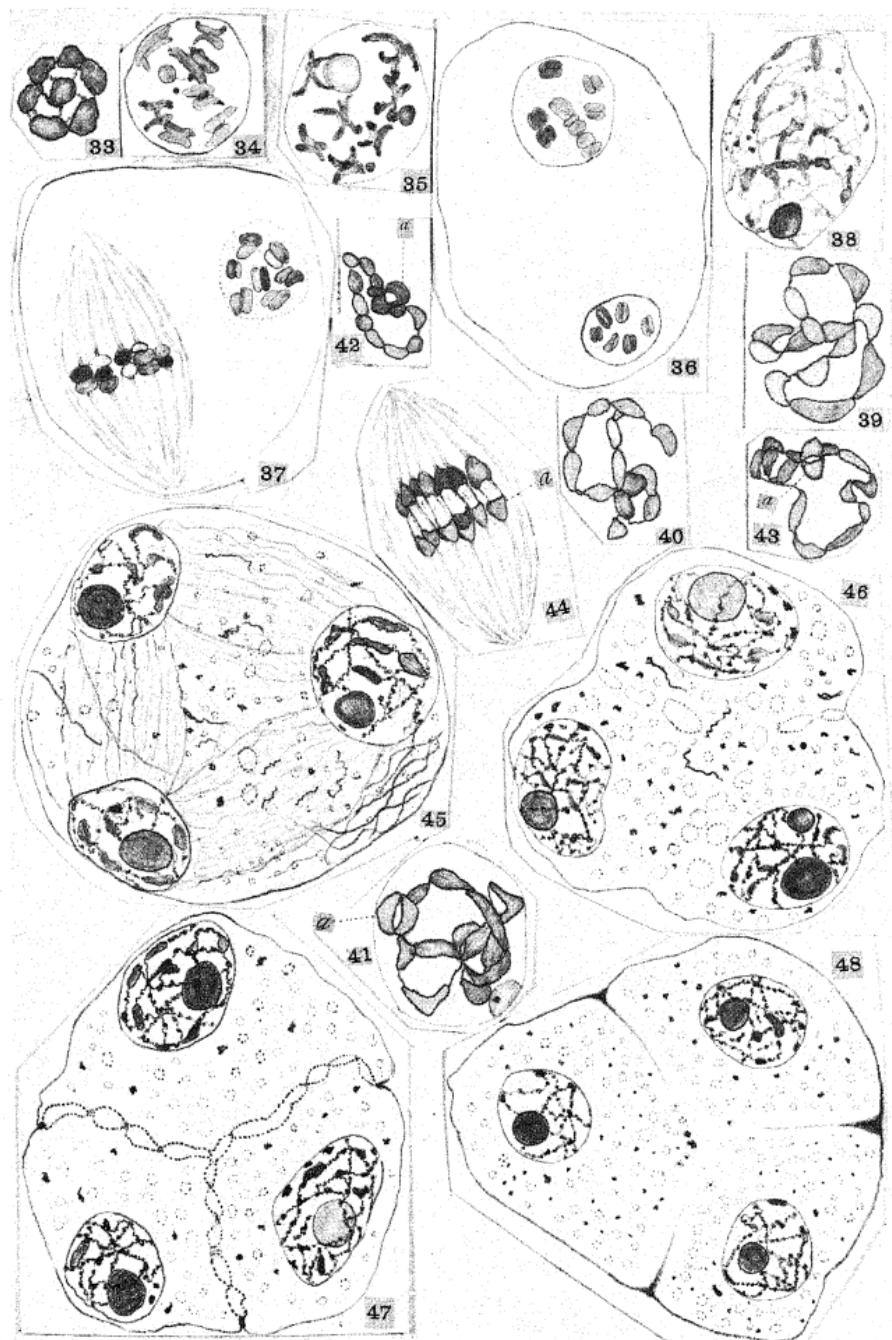


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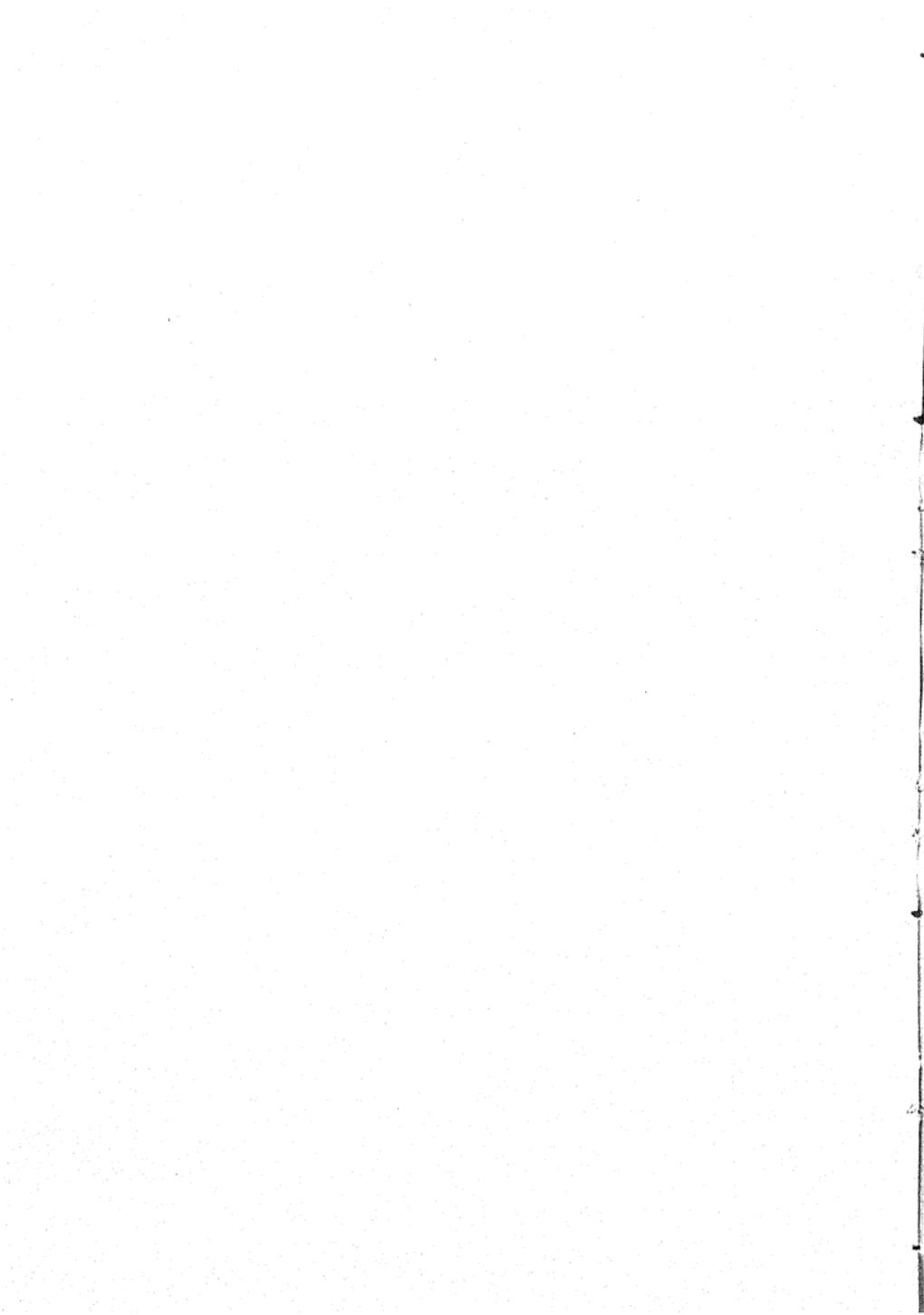


FIG. 43.—Same as fig. 42.

FIG. 44.—Metaphase of heterotypic mitosis in strain M; chain of twelve chromosomes and ring of two (*a*) at side of spindle separate from chain.

FIG. 45.—Late telophase of homeotypic mitosis; boundaries of some of chromosomes still visible; note spindle fibers connecting nuclei.

FIG. 46.—Late telophase; small refractive vacuoles in cytoplasm; wall of pollen mother cell infolded between nuclei.

FIG. 47.—Large flattened vacuoles formed by fusion of vacuoles between nuclei from center of pollen mother cell to furrows at periphery.

FIG. 48.—Furrows cutting inward from periphery toward center of pollen mother cell.

A REVISION OF THE GENUS COLLINSIA (SCROPHULARIACEAE)

VESTA MARIE NEWSOM

Introduction

In 1924, ALICE McCULLY MUNZ undertook a study of the southern California species of *Collinsia*. From her work it soon became evident that the genus as a whole needed revising, the most recent complete treatment being that of GRAY (Syn. Fl. N. Am. 2:255-257, supplement 439, 1886). The next year DR. PHILIP A. MUNZ of Pomona College, while working in the library of the Gray Herbarium, got together notes and copied species descriptions for the group as a whole. It was at his suggestion and under his direction that, in the fall of 1925, the present paper was undertaken.

In addition to expressing appreciation to Dr. MUNZ for his help and direction, I wish to acknowledge with gratitude the loan of material and supplying of information by the following: Dr. B. L. ROBINSON and Dr. IVAN M. JOHNSTON of the Gray Herbarium, Dr. W. L. JEPSON of the University of California, Dr. F. W. PENNELL of the Philadelphia Academy of Natural Sciences, Dr. L. R. ABRAMS of Stanford University, and Dr. HAROLD ST. JOHN of the State College of Washington. To Mrs. ALICE McCULLY MUNZ and Mr. DAVID D. KECK I express my thanks for field color notes of several species.

In citing herbarium material the following symbols are used: Gray Herbarium of Harvard University (G), Dudley Herbarium of Stanford University (S), Jepson Herbarium (J), Pomona College Herbarium (P).

History of treatment of genus

The genus *Collinsia* was first described by NUTTALL (Jour. Acad. Philad. 1:190. t. 9. 1817). He had collected *Collinsia verna*, the type species, as early as 1810 along the Allegheny River and Lake Erie, but the specimens were lost. In 1816 he again found it in seed at Gallipolis, Ohio, and based his generic and specific descriptions on

the plants raised from the seeds collected. The genus was dedicated to ZACCHEUS COLLINS of Philadelphia, who was a "very worthy botanist," but published nothing. RAFINESQUE (Am. Mo. Mag. 4: 194. 1819; Cincin. Lit. Gaz., 84. 1824) stated that Dr. MILLER of Pennsylvania was the first to find *Collinsia*, but that MUHLENBERG in classifying it mistook it for a new species of *Herpestis*. In the latter publication RAFINESQUE adds two new species, makes eight new varieties, and later (Cat. 13) adds a fourth species. DOUGLAS (LINDL., Bot. Reg. t. 1082 and t. 1107. 1827) was the first to publish far-western species, naming *C. pariflora* and *C. grandiflora* respectively. He sent seeds of another western species to BENTHAM, who grew them and then described *C. bicolor* (Trans. Hort. Soc. n. ser. 1:480. 1834). About this time, NUTTALL published two more species, *C. minima* (Jour. Acad. Philad. 7:47. 1834) and *C. violacea* (Trans. Am. Phil. Soc. 5:179. 1837). FISCHER and MEYER (Ind. Sem. Hort. Petrop. 2:33. 1836) named *C. sparsiflora*, and R. GRAHAM *C. heterophylla* (HOOK., Bot. Mag. t. 3695. 1838). WALPERS (Repert. 3:231-2. 1844-45) was the first to list the known species, and BENTHAM (DC., Prod. 10:318-19, 593. 1846) made the first comprehensive study of what was then a comparatively small genus. He added two new species.

New discoveries were made from time to time, but no further study of the group as a whole was made until ASA GRAY worked over the California species then known (Bot. Calif. 1:552-555. 1876), and later published the last complete treatment of the genus (Syn. Fl. N. Am. 2:255-57, supplement 439. 1886). Since then there have been many treatments in manuals covering restricted areas, and several new species and varieties have been added. The only recent study that is of real importance, however, is that made by JEPSON (Man. Calif. 902-905. 1925), which is a treatment of Californian plants only.

There has been a total number of forty-seven species published for the genus, and of this number seventeen species and twelve varieties are being recognized in this paper.

The genus *Collinsia* is very distinctly a unit, although it has on occasion been confused with *Tonella* (BENTH., in DC., Prod. 10:593. 1846; GREENE, Pittonia 1:55. 1887), but the presence of the keel-

shaped middle lobe of the lower lip in *Collinsia* readily separates the two. Aside from *Tonella*, *Collinsia* seems to have no other close relatives.

There is little evidence of specialization within the genus, except with reference to seed characters, which are discussed later. Generally speaking, the species all seem to be at about the same level of development, none being particularly primitive or specialized. However, they can quite easily be arranged in series. The length of the pedicels forms the basis for the most conspicuous division of the genus into the sessile- and pedicel-flowered groups, and these in turn are easily arranged in series according to bearding of stamen filaments or size and shape of corollas. ASA GRAY was the first to use the character of long and short pedicels in keying the species, and it has been used for the main grouping in all subsequent studies.

As to geographical distribution, all except two species occur in California, but they are not all restricted to this state. Only three species occur east of the Rocky Mountains. The sessile-flowered species are restricted to California and Lower California, while the pediceled ones range from east to west coast and from Lower California into Canada. *C. parviflora* is the most widely distributed species, occurring from the Rocky Mountains westward to the coast and from Canada to southern California, and eastward to Lake Superior.

Diagnostic characters for species

In searching for workable units, such gross characters as leaf size and shape, calyx shape, and the pubescence of the whole plant all have been found variable within a given species and thus not of much importance in a taxonomic study. With the exception of the long- and short-pedicled character, all the most important specific characters refer to the corolla, the stamens, and the seeds.

Corolla size may or may not be variable. In the *torreyi* group it is very constant in each variety, while in the *sparsiflora* group numerous intergradations, both in size and in shape of corolla, make the series a difficult one with which to work. In the large-flowered sessile forms variation in the length of the upper lip is a constant and workable character.

Variation in corolla color I am entirely disregarding, because

if such were considered, innumerable varieties would have to be made. For instance, *C. bicolor* var. *typica* has every intermediate shade between white or pale pink to very deep violet-blue. In *C. bartisiaefolia* var. *typica* there is intergradation from white to rose-lavender. These are closely allied shades, and when not consistent in variation do not seem worthy of taxonomic recognition. The revision must be based on more fundamental and constant structural characters.

Bearding on the lower half of filaments (usually on just the upper pair), although not always easily discernible, is most helpful in making some rather large and important groupings in comparison with species with glabrous stamen-filaments.

The size and shape of the seeds are important in numerous cases. There are two extremes in seed development. Species which have flattened and often winged or cup-shaped seeds when mature are found in all the sessile-flowered group, and in *sparsiflora*, *rattanii*, and *franciscana* of the long-pediceled group. Those seeds which become thickened and bean-shaped when mature characterize all the long-pediceled forms except those just mentioned. The seeds of the *rattanii* group and of *franciscana* really indicate an intermediate position between the two groups, however, generally not being thickened enough to belong to the second group, and yet not thin-margined enough for the first. The thickened seed is evidently the most highly developed, because the seeds of all the species are at least somewhat flattened when immature. If it were not for the intermediate groups, this seed character would offer a very good means of sub-generic division, but I do not feel that it is definite enough.

Description of genus

COLLISSIA.—Jour. Acad. Philad. 1:190, t. 9. 1817; Rafin., Cincin. Lit. Gaz. (for Neophyton 2), 84. 1824; Endl. Genera Pl. 3897. 1840; Benth., in DC. Prod. 10:317. 1846; Benth. & Hook. Genera Pl. 2nd:941. 1876; Gray Syn. Fl. N. Am. 2:246, 255. 1878; Baillon Hist. de Pl. 9:436. 1888; Gray Man., ed. II: 380. 1889; Engler-Prantl Pflan. Fam. 4, IIIb:64. 1895; Howell Fl. N.W. Am. 1:505. 1901; Britton & Brown Fl. N. U.S. & Can. ed. II, 3:188. 1913; Jepson Man. Calif., 901. 1925.

Annual herbs, 5-50 cm. tall, erect, decumbent or loosely branched, glabrous to puberulent or glandular-pubescent. Leaves opposite or whorled, petioled below to sessile above, entire or occasionally three- to five-parted below. Pedicels bracteate to ebracteate, short and almost sessile to long and filiform, one-flowered. Flowers in two- to eight-flowered whorls, forming a raceme with each whorl, cymose with pedicels not evident, or solitary and axillary. Calyx of five united sepals, campanulate, cleft; lobes sub-equal, the two lower ones broader and slightly shorter, the uppermost somewhat folded and appressed to the tube when tube is saccate. Corolla of five united petals, two-lipped, papilionaceous; corolla-tube straight or recurved, tubular to gibbous or saccate near base dorsally; upper lip two-lobed, with a transverse ridge or two-crested region within from which the lip is reflexed; lower lip three-lobed, the lateral lobes spreading-pendulous, middle lobe conduplicate into a keel-shaped sac which incloses the four declined stamens and style; keel coalesced one-half to one-third its length with lateral lobes to form an inconspicuous infolding saccate region descending into the tube as far as the lip divisions. Filaments four, didynamous, long and filiform, the lower or anterior pair inserted higher on the tube than the upper pair and somewhat shorter. Anthers round-reniform, the two cells confluent at the apex into one, 0.5-1 mm. wide, closely infolded with the stigma near the tip of the keel (or rarely exceeding the keel in old corollas). Rudimentary stamen or gland conical to oblong, located at the base of the corolla tube dorsally. Style long and filiform, persistent. Stigma small, capitate-entire to two-cleft. Capsule ovate or globose, glabrous, or rarely slightly pubescent, septicidal; valves two-cleft. Seeds amphitropous, peltate, dorsally convex, concave ventrally, flattened and winged to rounded and not winged, smooth to reticulate, two to many.

Type species for genus: *C. verna* Nutt.

Key to species

A. Flowers sessile, congested in whorls, with pedicels shorter than (to no longer than) the calyces in the lower whorls; seeds flattened when mature.

- B. Upper pair of filaments with no basal appendage, or with a rudimentary one present less than 1 mm. long.
- C. Upper lip of corolla conspicuously shorter than lower, or its sinus not over 2 (3) mm. deep.
- D. Filaments (at least the upper ones) well bearded; upper corolla lip with transverse but no conspicuous lateral callous crests at throat; pedicels of whorls shorter than calyces.
- E. Dorsal region of tube saccate, conspicuous, 3-4 mm. deep, short gland-bearded within; lateral corolla lobes usually long-bearded subcentrally, and longer and wider than keel; seeds 4, 2-3 mm. long; keel 1-2 mm. shorter than lateral lobes; leaves triangular-lanceolate, usually over 4 cm. long.....1. *C. tinctoria*
- E. Dorsal region of tube gibbous, inconspicuous, ca. 1 mm. deep, long-bearded within; lateral corolla lobes not bearded subcentrally, and equaling the keel in size; seeds more than 4, 1-1.5 mm. long; keel as long as lateral lobes; leaves oblong, crenate, not over 3 cm. long.
- F. Flowers over 13 mm. long, in dense capitate clusters or whorls; plant rather stout, branching, diffuse, almost decumbent (occasionally erect); veining of corolla lobes not conspicuous. Mendocino County to vicinity of San Francisco, California. 2. *C. corymbosa*
- F. Flowers less than 10 mm. long, in several slender whorls; plant slender, erect; veining of corolla lobes evident. Monterey and San Benito Counties and Sierran California.....3. *C. bartsiaeifolia*
- D. Filaments glabrous, the upper pair bearded at base; upper corolla lip with distinct lateral and transverse callous crests projecting into throat; pedicels of lower whorls as long as calyces.....6. *C. greenei*
- C. Upper lip of corolla as long as or slightly shorter than lower lip, its sinus more than 2 mm. deep; plants erect.
- D. Leaves thin, serrulate-entire; corolla lavender-blue, appearing truncate, veining of lobes not evident. Foothills of San Bernardino Mts. south to Lower California

4. *C. concolor*

D. Leaves thick, crenate; corolla white to rose-lavender, lobes broad and rounded, veins evident and usually somewhat darkened.....3. *C. bartisiaefolia*

B. Upper filaments with a distinct basal appendage 1-2 mm. long

5. *C. bicolor*

A. Flowers pediceled, solitary or in whorls; pedicels of lower corollas from as long as to longer than calyces; seeds thickened or flattened.

B. Upper filaments bearded (occasionally very sparsely so in no. 11).

C. Plants from east of Rocky Mountains (corolla over 8 mm. long).

D. Seeds normally 4, 2-3 mm. long; upper lip of corolla white or tinged lavender, almost equaling lower lip in length; lower lip bright blue; corolla lobes retuse; calyx rather pale, submembranous below; leaves thin, ovate to deltoid-lanceolate. New York to Missouri.....8. *C. verna*

D. Seeds 6-12, 1-1.5 mm. long; corolla bright violet, upper lip slightly paler and conspicuously shorter than lower lip; corolla lobes obtuse, notch acute, 1 mm. deep; calyx purplish, inconspicuously submembranous below; leaves thickened, oblong to lance-oblong. Southeastern Kansas, southwestern Missouri to Texas..9. *C. violacea*

C. Plants from west of Rocky Mountains (corolla 4-6 mm. long, except in no. 12).

D. Plants from central California and northward; mature seeds thin and flattened, winged, 2-3 mm. long.

E. Inflorescence glandular-pubescent, often viscid; cauline leaves deltoid-ovate to lance-ovate; corolla 12-18 mm. long; upper lip white, lower bluish-purple. San Francisco, San Mateo, Santa Clara counties

7. *C. franciscana*

E. Inflorescence glabrous to sparse-pubescent; cauline leaves oblong to lanceolate; corolla whitish-lavender to purple.....10. *C. sparsiflora*

D. Plants of southern California; mature seeds thick, not winged, 1 mm. long; upper stem and pedicels finely retrorse-pubescent.....11. *C. parryi*

- B. Upper filaments glabrous, except for some bearding at place of attachment.
- C. Bracts at least 2 mm. long in upper whorls of inflorescence (in number 15, the uppermost bracts may be shorter).
- D. Upper lip of corolla with no conspicuous lateral and transverse callous crests projecting into throat.
- E. Dorsal side of tube saccate when corolla is over 7 mm. long, or at least gibbous when corolla is less than 7 mm. long; corolla throat closed by the declined callous ridge of upper lip; calyx lobes subulate-acuminate; inflorescence glabrate to pubescent or inconspicuously glandular-pubescent; seeds 4.
 - F. Corolla 4–6 mm. long, white and blue to violet-blue; corolla tube gibbous at base, erect or slightly declined; corolla lips inconspicuously divergent, $\frac{1}{3}$ the total corolla length, lobes narrowed and entire; calyx over half the length of corolla, its lobes somewhat concealing the corolla tube.....12. *C. parviflora*
 - F. Corolla over 6.5 mm. long, violet; corolla tube deeply saccate at base, strongly declined to form a right angle with pedicel; corolla lips divergent, $\frac{1}{2}$ – $\frac{1}{3}$ the corolla length, lobes broad in appearance, retuse or emarginate; calyx $\frac{1}{3}$ – $\frac{1}{2}$ the corolla length, its lobes not concealing the corolla tube.....13. *C. grandiflora*
- E. Dorsal side of tube merely gibbous when corolla is over 7 mm. long, tubular when corolla is less than 7 mm. long; corolla throat open, callous ridge not declined into the throat; calyx lobes linear to subulate (acuminate), obtuse to rather broadly acute; inflorescence conspicuously glandular-(viscid-) pubescent (no. 16 variable).
 - F. Cauline leaves linear, thickened, gray-green. Northern California to Washington.....14. *C. rattanii*
 - F. Cauline leaves ovate to oblong. Southern and central California.
 - G. Seeds 2, ca. 3 mm. long; leaves thin; calyx membranous, rounded and ca. 3 mm. wide at base in fruit, lobes exceeding the capsule.....15. *C. childii*

- G. Seeds 6-8, ca. 2 mm. long; calyx and leaves thick and succulent; calyx squared and swollen, ca. 5 mm. wide at base in fruit, lobes about equaling the length of the capsule.....16. *C. callosa*
- D. Upper corolla lip with conspicuous lateral and transverse callous crests projecting into throat. North coast ranges, California6. *C. greenei*
- C. Bracts of inflorescence obsolete, or minute and less than 2 mm. long in upper whorls (leafy bracts ca. 1 cm. long when plants are immature or 1-2 flowered, otherwise typical); corolla throat open; seeds 2; calyx never membranous below, lobes not longer than the mature capsule....17. *C. torreyi*

Treatment of species

1. *COLLINSIA TINCTORIA* Hartw., in Benth. Pl. Hartw., 328. 1848; Gray Syn. Fl. No. Am. 2:255. 1878; Jepson Man. Calif., 902. 1925; *C. barbata* Bosse, Verh. Berl. Gartenb. Ver. N. Reihe 1:399. 1853; *C. septemnervia* Kellogg, Proc. Cal. Acad. 2:224. 1863.

Plant 20-60 cm. high; stems simple, erect, or occasionally sparsely branched, glabrous below, glandular-viscid above, imparting a brownish stain; leaves thin, ovate to broadly triangular-lanceolate, acute, serrate-crenate to entire, scabrous, sessile by a broad or subcordate base, glabrous to sparsely puberulent above, puberulent below, 1-10 cm. long, 0.5-4.0 cm. wide; lower leaves oblong, obtuse, with petioles 5-10 mm. long; flowers in 2-6 many-flowered whorls, sessile or with pedicels not over 2 mm. long; bracts linear, entire, glandular-pubescent, 5-10 mm. long, ca. 1 mm. wide; calyx deeply parted, submembranous at base, 5-8 mm. long; calyx lobes linear to lanceolate, obtuse to broadly acute, densely glandular-pubescent, 4-7 mm. long, ca. 1 mm. wide; corolla strongly declined, yellow to greenish white, with purple dots or lines, 12-16 mm. long; corolla tube 5 mm. long, ca. 2 mm. wide at base, abruptly widened by the dorsal saccate region; saccate region short gland-bearded within, 3-4 mm. deep at base, forming a right or obtuse angle with the tube; upper lip 4-5 mm. long, ca. 2 mm. of the lip length reflexed from the transverse calloused ridge; lobes rudimentary, emarginate, sinus less than 1 mm. deep; lower lip 5-10 mm.

long, keel 1-2 mm. shorter than the lateral lobes; lateral lobes ovate-spatulate, obtuse, entire, long-bearded within at subcentral region, 4-5 mm. wide; keel sparsely gland-bearded without, 2-3 mm. deep; lateral inverted saccate region sparsely gland-bearded within; upper pair of filaments bearded half their length; rudimentary basal appendage not over 0.8 mm. long, bearded; lower filaments rarely bearded; anthers brick red; stigma not lobed; capsule 3-4 mm. long, well exceeded by calyx lobes; seeds flattened, round-oblong, rugose-reticulate without, smoother within, slightly winged, 2-3 mm. long, 1-2 mm. wide, normally 4 in number.

Type locality.—"In montibus Sacramento."

Representative material.—CALIFORNIA: without locality, *Hartweg* 1882, in 1846-47, type collection (G); between McCloud and Sacramento rivers, *Heller* 12498 (S); Table Mountain, 8 miles north of Oroville, *Heller* 10768 (S); Little Chico, *Bruce* 1845 (P); Mt. Diablo, North Peak, *Jepson* 7612 (S), on road to summit, *Abrams* 8019 (S); Sonoma Creek at the foot of Mt. Hood, *Heller* in 1902 (S); Eshom Valley, *Clemens* in 1910 (P); vicinity of Homer's Nose, Sequoia National Park, *Dudley* 1811 (S); South Fork of Kings River, *Clemens* in 1910 (P); Collins Meadow, *Hall & Chandler* 459 (P, S); Yosemite Valley, Kenneyville, *Abrams* 4554 (S); Yosemite Valley, *M. E. Jones* in 1884 (P); the trail from Tioga Road to Yosemite Falls, *Munz* 7518 (P); Mather, *Munz* 7367 (P); Longworthy's near North Fork, Sierra National Forest, *Abrams* 4966a (S); Big Trees, Calaveras Co., *Dudley* in 1906 (S); Kelsey, El Dorado Co., *M. E. Jones* in 1883 (P).

This species, while it has considerable variation in the amount of pubescence and in leaf length, is constant as to corolla and seed characters. Plants from two localities are exceptions to the description given for the species, but do not seem to justify varietal rank. Material cited from Mt. Diablo, while typical in floral structure, has thickened and rather succulent leaves, due probably to ecological conditions. It is also often dwarfed in growth, for example, *Jepson* 7612 (S) is only 10 cm. high. *Munz* 7518 from the Tioga trail to Yosemite Falls (P) is peculiar in that the lateral lobes have no subcentral bearding; the lobes of the upper lip are rather larger and more conspicuous than ordinary, and the dorsal saccate region is less conspicuous than in typical material.

2. *COLLIINSIA CORYMBOSA* Herder, Ind. Sem. Hort. Petrop., 32. 1857; Regel, Gartenfl. 33: t. 568. 1868; Gray Syn. Fl. No. Am. 2:

255. 1878; Jepson Man. Calif., 903. 1925; *C. bartsiaeifolia* of Jepson, *l.c.*, in part.

Diffusely branched, almost decumbent, occasionally simple and erect; branches finely white-pubescent to glabrate, infrequently sparsely glandular, 10-18 cm. long; leaves rather fleshy, oblong to ovate or elliptical, obtuse, crenate, villous to glabrate above, glabrous below, 10-20 mm. long, 5-10 mm. wide; flowers sessile or with pedicels not over 5 mm. long, in whorls of 3 to 10, these either crowded in a terminal inflorescence, appearing corymbose, or 1 cm. or more apart; bracts crenulate, sparsely to densely villous-pubescent, 5-9 mm. long, 3-5 mm. wide; calyx puberulent to conspicuously villous-pubescent, "whitish green," 7-10 mm. long; calyx lobes oblong to broadly ovate, obtuse, 3-5 mm. long, 1.5-2 mm. wide; corolla strongly declined, pale lavender to yellowish-white, long and appearing tubular, the outspread lobes not over half the total corolla length, 13-20 mm. long; corolla tube 7-10 mm. long, 3-5 mm. wide; dorsal gibbous region of tube shallow, 1 mm. deep, bearded within, the bearding becoming longer toward the top; upper lip blue, 3-7 mm. long, the lobes yellowish, reflexed, from apiculate and almost obsolete to truncate and 4-6 mm. long; the sinus 1-2 mm. deep; lower lip 10-20 mm. long; lateral lobes as long as the keel, entire or retuse, broadly spatulate, 4-6 mm. wide, somewhat divergent; keel 3-5 mm. deep; upper pair of filaments bearded for half their length; lower filaments more sparsely bearded; stigma not lobed; capsule 5-6 mm. long, exceeded by the calyx lobes; seeds flattened, oblong, many, irregularly winged, finely rugose-reticulate, 1-1.5 mm. long.

Type locality.—"from Mexico" according to Regel, *l.c.*, but this is certainly incorrect, since the range is restricted to the north-central coast of California.

Material studied.—CALIFORNIA: without locality, Samuels (G), Dr. Gibbons in 1853 (G), Kellogg & Harford 660 (G); Ft. Bragg, Bolander 4783 (G); Bolenas Bay, Bigelow in 1849 (G); San Francisco, Jones in 1882 (P), Greene 161 (G); Mt. Lake, San Francisco, Eastwood in 1894 (G); Lake Merced, S.F. Co., Abrams 1601 (S); Presidio, S.F., Dudley in 1902 (S), Baker 710 (P), Michener & Bioletti 77 (G), Tidestrom in 1895 (P).

This species is amply distinct from *C. bartsiaeifolia* var. *typica*, with which it has been confused, in having a shorter upper lip, corolla lobes not evidently veined, and the corolla appearing tubular. Typical *corymbosa* has a very short apiculate upper lip (for example,

Bolander 4783 and *Baker* 710), but this character is variable, and in material from the same locality as the *Baker* collection are found all intergradations from apiculate lobes ca. 1 mm. long to truncate lobes, with a sinus between them ca. 2 mm. deep (*Jones* in 1882). Because of the scarcity of material and the limited range, it is thought best not to make a separate variety of the form with the longer upper lip.

3. *COLLINSIA BARTSIAEFOLIA* Benth. in DC. *Prodr.* 10:318. 1846.

Stems erect, or diffusely branching, puberulent below, villous and often glandular above, 10-35 cm. long; leaves somewhat erect, oblong to lance-oblong, obtuse, crenate, rarely entire, scabrous-margined, thickened, sparsely villous, 1-4 cm. long, 0.5-2 cm. wide; flowers sessile or with pedicels not over 4 mm. long, in several-flowered whorls; bracts ovate to linear, serrulate to entire, glabrate to long ciliate-pubescent, 3-10 mm. long, 1-5 mm. wide; calyx ciliate-pubescent to villous-pubescent throughout, 4-10 mm. long; calyx lobes lance-oblong, obtuse to acute, 1.5-6 mm. long, 1-3 mm. wide; corolla rose-purple to lilac or almost white, the veining darker colored and conspicuous, slightly declined, 7-20 mm. long; corolla tube 2.5-5 mm. long, 2-4 mm. wide; dorsal gibbous region shallow, 1-1.5 mm. deep, long-bearded within; upper lip paler than lower, purple-dotted below the sinus, 3.5-12 mm. long, the lobes rectangular, serrate-emarginate to entire, the sinus 1-5 mm. deep; reflexed region of upper lip 1.5-9 mm. long; lower lip 5-14 mm. long; keel 2 mm. shorter than lateral lobes, pale with brown-purple dots within at tip, 2-4 mm. deep; lateral lobes round-spatulate, emarginate to entire, 6-9 mm. wide, divergent; upper pair of filaments bearded to above the middle with bearding especially dense at place of attachment and on the very short appendage which is ca. 0.5 mm. long if present; lower filaments glabrous or bearded for one-third their length; stigma oblong but not definitely lobed; capsule 3.5-5 mm. long, scarcely exceeded by the calyx lobes; seeds many, flattened, rounded, slightly rugose-reticulate, 0.7-2 mm. long.

Key to varieties

A. Lateral lobes of corolla emarginate, conspicuously wider than keel; sinus at least 2 mm. deep; flowers 9-20 mm. long; plant stout in appearance.

B. Corolla 15-20 mm. long; plant 15-35 cm. tall; seeds not over 1 mm. long. Central California

3a. *C. bartisiaefolia* var. *typica*

B. Corolla 9-13 mm. long; plant 7-20 cm. tall; seeds ca. 1.5 mm. long. North and west edges of Mojave Desert

3b. *C. bartisiaefolia* var. *davidsonii*

A. Lateral lobes of corolla entire, scarcely wider than keel; sinus less than 2 mm. deep; plant slender; seeds 1.5 mm. long

3c. *C. bartisiaefolia* var. *stricta*

3a. *COLLINSIA BARTSIAEFLORIA* var. *typica*, nom. nov.—*C. bartisiaefolia* Benth., in DC. Prodr. 10:318. 1846; in Pl. Hartw., 328, no. 1885. 1839-1857; Gray Syn. Fl. No. Am. 2:255. 1878; Jepson Man. Calif., 903. 1925 (in part); *C. bartisiaefolia* var. *alba* Orcutt, West. Amer. Sci. 7:126. 1891; *C. hirsuta* Kellogg, Proc. Cal. Acad. 2:110, t. 34. 1863.

Plant 15-35 cm. tall; corolla 15-20 mm. long; seeds small, not over 1 mm. long, restricted to central California.

Type locality.—Given by BENTHAM as "in montibus scopulosis," but the material collected by Fremont was all from California, probably the San Joaquin Valley.

Representative material.—CALIFORNIA: without locality, but without doubt from San Joaquin Valley, Fremont in 1844, type collection (G), in 1846 (G); Lakeport, Bentley in 1917, in part (S); Antioch, Contra Costa Co., Rattan in 1880 (S); Tracy, San Joaquin Co., Baker 2783 (P); Riverbank, Stanislaus Co., Abrams 9963 (S); 5 miles above Pollasky, Madera Co., Heller 8157 (S); Raymond, Madera Co., Cummings in 1896 (G); near Fresno, Dudley in 1900 (S); north of Bakersfield, Cooper in 1927 (P); Tulare Co., Kaweah, Eastwood in 1895 (G), Ducor, Munz 9041 (P); Del Monte, Monterey Co., Heller 6666 (P, S).

This plant is quite distinct from *bicolor* in its lack of long stamen appendages and in the conspicuous veining of the corolla lobes. Specimens from Keene, Jones in 1903 (P), and Fort Tejon, de Vesey 59 (G), may be cited as var. *typica* approaching var. *davidsonii*. They have the appearance of *davidsonii* but the seeds of *typica*.

3b. *COLLINSIA BARTSIAEFLORIA* var. *davidsonii* (Parish), comb. nov.—*C. davidsonii* Parish, Zoe 4:147. 1893; Jepson Man. Calif., 903. 1925.

Plant smaller, not over 20 cm. high; stems often glabrate; flower 9-11 mm. long; seeds ca. 1.5 mm. long, often becoming slightly cup-shaped.

Type locality.—Lancaster, Mojave Desert.

Material studied.—CALIFORNIA: "Southern California," *Parry & Lemmon* 294 (G); Mojave Desert, *Pringle* in 1882 (G); Tejon Pass road, Mt. Piños region, *Dudley & Lamb* 4447 (S); Elizabeth Lake Canyon, Liebre Mts., *Dudley & Lamb* 4414 (S), west of Elizabeth Lake, *Pearson* in 1922 (P); ten miles south of Willow Springs, *Munz* 9994 (P); Antelope Valley, *Davidson* in 1895 (S), Lancaster, *Davidson* in 1893, type collection (G), *Dudley & Lamb* 4319 (S); Mojave River, near Hesperia, *Parish* 4984 (S), Los Flores Rancho, *Saunders* in 1906 (S).

This plant, which has heretofore been considered as a distinct species, seems to me to be only a small desert variety. Aside from the smaller habit, the main character that makes it of good varietal rank is the increased size of the seeds; otherwise, the morphology of the two varieties is identical.

3c. *COLLINSIA BARTSIAEOLIA* var. *stricta* (Greene), comb. nov.—*C. stricta* Greene, *Pittonia* 2:23. 1889; *C. tinctoria* var. *stricta* (Greene) Jepson Man. Calif., 903. 1925; *C. bicolor* var. ?, Benth. in *Plantae Hartw.*, 328., no. 1884. 1839-57.

Resembling *typica* but more slender; flowers smaller, 8-9 mm. long; lateral lobes of corolla not emarginate and not much wider than keel; upper lip narrow, ca. 4 mm. long, sinus less than 2 mm. deep; seeds numerous, ca. 1.5 mm. long, often slightly cup-shaped.

Type locality.—"Bushy hills near Sheep Ranch, Calaveras Co."

Material studied.—CALIFORNIA: Calaveras Co., *Greene* in 1889, type collection (S); "in montibus Sacramento," *Hartweg* 1884 (G); Plumas Co., *Ames* in 1875 (?) (G); San Antonio Mission, Monterey Co., *Dudley* in 1895 (S); New Idria, San Benito Co., *Dudley* in 1899 (S).

Although this has been considered as a separate species by *GREENE* and as a variety of *tinctoria* by *JEPSON* because of the gibbous shape of the tube and the veining of the petals, it belongs very distinctly with *bartsiaeolia* in my opinion, even though the narrow lip is peculiar and variable. The general appearance of the plant, the distinct veining of the petals, and the shape, size, and number of the seeds suggest *bartsiaeolia*.

4. *COLLINSIA CONCOLOR* Greene, *Erythea* 3:49. 1895; *C. bicolor* var. *concolor* (Greene) Jepson Man. Calif., 902. 1925.

Stems erect, occasionally diffusely branched, puberulent to glabrate, rarely glandular, 15-45 cm. tall; leaves thin, lanceolate-oblong, narrowly obtuse to acute, puberulent to glabrate, serrulate

and revolute to entire, scabrous-margined, cordate at base, 10-50 mm. long, 2-12 mm. wide; lower leaves smaller with petioles 2-20 mm. long; flowers sessile or with pedicels not over 4 mm. long, in several-flowered whorls; bracts linear, entire, puberulent to long ciliate-pubescent, 5-10 mm. long, 1-2 mm. wide; calyx scabrous-margined, pubescent above to villous-pubescent and submembranous below, 6-8 mm. long; calyx lobes oblong, broadly acute, 3-4 mm. long, ca. 1 mm. wide; corolla declined, appearing squarish-truncate, bluish lavender, 12-15 mm. long; corolla tube 3-5 mm. long, 3-4 mm. wide; dorsal gibbous region shallow, ca. 1.5 mm. deep, long-bearded within, especially toward the base; upper lip pale lavender to blue, 6-10 mm. long, reflexed from the 2-crested callous region; sinus 3-4 mm. deep, lobes truncate, emarginate, 3-4 mm. wide; triangular region between sinus and callosity spotted red-purple; lower lip deep lavender-blue, 8-11 mm. long; keel paler and yellowish at tip, sparsely bearded without, 2.5-4 mm. deep, ca. 1.5 mm. shorter than the lateral lobes; lateral lobes broadly spatulate, emarginate, divergent, sparsely bearded within, 3-6 mm. wide; upper pair of filaments bearded half way up, often with a rudimentary bearded basal appendage 0.5 mm. long; lower filaments rarely sparsely bearded near base; stigma not lobed; capsule 4 mm. long, usually well exceeded by calyx lobes; seeds flattened, irregularly round, rugose, ca. 1.5 mm. long, many in number (8-16).

Type locality.—"Witch Creek, San Diego Co."

Some of specimens studied.—CALIFORNIA: Yucaipa, San Bernardino Co., *Craig, Newsom, & Hilend* 367 (P); Idyllwild, Riverside Co., *Spencer* 2166 (P); Hemet Valley, *Munz & Johnston* 5532 (P); 20 miles N. of Idyllwild, *Jones* in 1926 (P); Dripping Spring, *Munz* 5100 (P); Palomar Mts., Oak Grove Trail, *Munz* 10395 (P); at Pine Hills, *Spencer* 373 (G,P); Warner's Hot Springs, *Munz* 9861 (P); Laguna Mts., *Munz* 8348 (P); Campbell Ranch, Laguna Mts., *Mearns* 3644 (S); Julian, *Brandegee* in 1894 (G,S); Witch Creek, *Alderson* in 1894, probably type material (G,S); Potrero Grade, *Munz & McCully* 8056 (P); Summit, W. of Jacumba, *Munz* 8043 (P); Campo, *Abrams* 3608 (S). LOWER CALIFORNIA: 45 miles S. E. of Tecate, *Munz* 9530 (P).

C. concolor has often been confused with *bicolor*, but it is easily distinguished from the latter by the lack of the long stamen appendage. If this is present at all, it is merely rudimentary. Then too, the flower of *concolor* has a square or truncate appearance, while in

bicolor the lobes are more rounded and spreading. The color of *bicolor* tends toward a lavender, that of *concolor* toward blue. In *bicolor* the region of reddish dots below the sinus of the upper lip varies from rounded to almost square, while in *concolor* it is triangular.

5. *COLLINSIA BICOLOR* Benth., Trans. Hort. Soc. N.S. 1:480. 1834.

Stems simple or diffusely branching, erect or somewhat reclining, glabrous to pubescent, occasionally sparsely glandular above, green to purplish, 10–50 cm. high; leaves green to reddish-purple, paler below, glabrous to pubescent, lanceolate-oblong, broadly acute, the base broad, margins serrulate to crenulate or entire, scabrous, 1–7 cm. long, 0.5–3 cm. wide; lower leaves more ovate, with petioles 2–10 mm. long; flowers sessile or with pubescent or often glandular pedicels 2–5 mm. long, in 2–7-flowered whorls; bracts lanceolate to linear, obscurely serrulate to entire, scabrous, glabrous to pilose-pubescent, 5–20 mm. long, 1–7 mm. wide, often becoming very leaf-like in lower whorls of inflorescence; calyx green to reddish-purple, submembranous, glabrous to long villous-pubescent, 5–10 mm. long; calyx lobes each 3-ribbed, linear to broadly lanceolate, narrowly obtuse to acute, glabrate (but scabrous-margined) to long villous-pubescent especially near base, occasionally sparsely glandular, 4–8 mm. long, 1–3 mm. wide; corolla declined, sparsely gland-bearded without, 1–2 cm. long; corolla tube 5–7 mm. long, 4–8 mm. wide; dorsal saccate region rounded, shallow, dark purple-lined, long bearded within, ca. 2 mm. deep; upper lip lilac to white, reflexed from the 2-crested transverse callosity, 3.5–12 mm. long, almost equaling to less than half as long as lower lip; upper lobes round-truncate, entire or retuse, often sparsely bearded within, 1.5–5 mm. wide, sinus 0.5–4 mm. deep, rounded region between the sinus and callosity spotted red-purple; lower lip violet or rose-purple to whitish, tips of lobes darker colored, sparsely bearded within, 6–14 mm. long; keel 1–2 mm. shorter than lateral lobes, 2–5 mm. deep; lateral lobes divergent, broadly spatulate, entire or emarginate, sparsely bearded within, 4–7 mm. wide; upper pair of filaments bearded half their length, possessing a linear bearded basal appendage ca. 2 mm. long and projecting into the base of saccate region of tube; lower filaments beardless; anther sacs dark purple; stigma not lobed;

capsule 4-7 mm. long, somewhat exceeded by calyx lobes; seeds flattened, ovate, rugose-reticulate, slightly winged, many in number, ca. 1.5-2 mm. long.

Key to varieties

A. Upper lip of corolla conspicuous, as long as or only slightly shorter than the lower lip; sinus of upper lip over 2 mm. deep

5a. *C. bicolor* var. *typica*

A. Upper lip of corolla short and recurved; sinus not over 2 mm. deep. Mountains of southern California

5b. *C. bicolor* var. *austromontana*

5a. *C. bicolor* var. *typica*, nom. nov.—*C. bicolor* Benth., Trans. Hort. Soc. N.S. 1:480. 1834; in DC. Prodr. 10:318. 1846; Curtis' Bot. Mag., t. 3488. 1838; Benth. Pl. Hartw., 328. no. 1883. 1839-57; Paxton's Mag. 3:195; Gray Syn. Fl. No. Am. 2:255. 1878; Jepson Man. Calif., 902. 1925; *C. heterophylla* R. Grah. in Hook. Bot. Mag. 65:t. 3695. 1838; *C. multicolor* Lindl. & Paxt., Flow. Gard. 2:89, t. 55. 1851-52.

Upper lip of corolla conspicuous, as long as or only slightly shorter than the lower lip; sinus of upper lip over 2 mm. deep.

Type locality.—CALIFORNIA.

Representative material.—CALIFORNIA: without locality, *Douglas*, probably type material (G), *Fremont* 442 (G), *Coulter* 602 (G); 20 miles north of Laytonville, Mendocino Co., *Munz* 9885 (P); Sulphur Banks, Lake Co., *Bowman* in 1902 (S); Lake Merced, San Mateo Co., *Elmer* 5046 (P,S); Santa Cruz, *Thompson* in 1902 (P,S); Stanford University, *Abrams* 2364 (P,S); foothills west of Los Gatos, *Heller* 7403 (S); New Idria, San Benito Co., *Abrams* & *Borthwick* 7968 (S); Monterey, *Eastwood* in 1900 (G); Point Piños near Pacific Grove, *Heller* 6578 (S); Paso Robles, *Munz* 10140 (P); Marysville Buttes, Butte Co., *Heller* 5559 (S); Caminetti Ranch near Jackson, Amador Co., *Mulliken* 101 (P,S); near French Flat, Tuolumne Co., *Ferris* 1510 (S); 3 miles from Oakdale, Stanislaus Co., *Abrams* 9967 (S); near Milo, Tulare Co., *Dudley* in 1900 (S); Los Angeles Co., South Hills near Pomona, *Munz* 2087 (P); Pasadena, *Jones* 3432 (P); Evey Canyon north of Claremont, *Johnston* in 1917 (P); San Gabriel Canyon, *Munz* 9410 (P); Lemon Tank, San Clemente Is., *Munz* 6686 (P); Sierra Canyon, Santa Ana Mts., *Munz* & *Harwood* 3747 (P); San Bernardino, *Parish* 263 (S); canyon near Murietta, Riverside Co., *Robinson* & *Crocker* in 1916 (P); above Escondido, *Munz* & *McCully* 8094 (P); San Diego, *Brandegee* 1709 (P); Witch Creek, *Abrams* 3782 (S). LOWER CALIFORNIA: without locality, *Jones* in 1882 (P); 10 miles north of Ensenada, *Canby* in 1925 (P); Coronado Is., *Cowles* in 1921 (P). MEXICO: Oaxaca, *Conzatti* in 1898 (G).

The *bicolor* group is very easily distinguished from all others by the long basal appendage to the upper filaments (cf. ALICE E. KEENER, BOT. GAZ. 20:232. 1895). As to size of corolla and leaves, and the amount of pubescence, *typica* is extremely variable. The length of bracts also varies. Material from Oaxaca, Mex., might be of good varietal rank, because of the large whorled bracts which make the corollas inconspicuous, but it is here included in *typica* because of lack of sufficient material for thorough study. The color of the corolla varies from almost entirely white to conspicuously lavender or deep purple, with the upper lip of the corolla usually paler.

The following specimens may be cited as intermediates between *typica* and *austromontana*.—Cobb Mt., Lake Co., *Leithold* in 1893 (S); Mt. Diablo, *Abrams* 8029 and 8055 (S); Petrified Forest, Sonoma Co., *Heller* in 1902 (S); near San Bernardino, *Parish* in 1901 (S), *Parish* 11216 (P); Los Angeles Co., hills above Claremont, *Jones* in 1926 (P); Little Santa Anita Canyon, *Abrams* 2613 (S); Mt. Lowe, *Diehl* 243 (P); hills near Pasadena, *Jones* in 1902 (P); San Diego Co., San Diego, *Dunn* in 1891 (S), *Fernald* in 1896 (G); Pine Hills, *Spencer* 1616 (P).

5b. C. BICOLOR var. *austromontana*, var. nov.

Labium superiore breve, reflexum; sinu non plusquam 2 mm. lato.

Type, near Brown's Flats, San Gabriel Mts., Los Angeles County, California, *Johnston* 1750 (Pomona College Herbarium no. 4314; cotypes at Pomona and Stanford).

Specimens studied.—CALIFORNIA: San Gabriel Mts., Lower San Sevaine Flats, *Johnston* 1750 (P), 4122 (P); on old road to San Sevaine Flats, *Johnston* in 1925 (P); Cucamonga Canyon, *Johnston* in 1919 (P), *Davis* in 1915 (P); Stoddard's Canyon, *Jones* in 1924 (P); Big Santa Anita Canyon, *F. Grinnell Jr.* in 1917 (S); San Bernardino Mts., south slope, *Parish* in 1895 (S); North Fork of Deep Creek, *Abrams* & *McGregor* 718 (S); Santa Ana Canyon, *Jaeger* in 1925 (P); Julian, San Diego Co., *McGregor* in 1918 (S).

Confused with *tinctoria* because of the short upper lip, var. *austromontana*, because of the long filament appendages, must be included in *bicolor*. Only the material with the extremely short upper lip is classed as *austromontana*, and the numerous intergradations between this and *typica* with the long upper lip are classed as intermediates, and have been cited under *typica*. True *austromontana* is restricted to the mountains of southern California, but some of the intermediates extend into the central part of the state.

6. *COLLINSIA GREENEI* Gray, Proc. Am. Acad. 10:75. 1874; Syn. Fl. No. Am. 2:256. 1878; Jepson Man. Calif., 903. 1925.

Stems slender, diffusely branching, puberulent to villous-pubescent and glandular, 10-30 cm. long; leaves oblong-linear, tapering to apex, broadly acute, dentate or denticulate, glabrous to puberulent, 7-30 mm. long, 2-7 mm. wide; lower leaves more ovate with petioles ca. 1 cm. long; pedicels 1-5 at a node, glandular-puberulent, 2-10 mm. long; bracts linear, sparsely dentate to entire, puberulent to glandular, 3-10 mm. long, ca. 1-2 mm. wide; calyx puberulent to glandular-pubescent, 4-7 mm. long; calyx lobes lanceolate, narrowly obtuse to acute, 2-4 mm. long, ca. 1 mm. wide; corolla "deep azure-blue," 10-12 mm. long; corolla tube ca. 6 mm. long, 4-5 mm. wide; dorsal gibbous region shallow, 1 mm. deep, short-bearded; throat open; upper lip shorter than lower, 4-5 mm. long, reflexed region ca. 2 mm. long, the lobes rounded, sinus 1 mm. deep and subtended by a two-crested transverse callous ridge, and extended down each side of lip forming conspicuous lateral crests, these crests ca. 1 mm. long and almost as wide; lower lip 5-6 mm. long; small lateral lobes scarcely as long as the keel, spatulate, ca. 5 mm. long, 1.5 mm. wide; keel 3 mm. deep; lateral saccate region sparsely gland-bearded within; upper pair of filaments glabrous or sparsely bearded at base; stigma not lobed; capsule 4 mm. long, just exceeded by calyx lobes; seeds few, normally 4, oblong, flattened or cup-shaped, smooth, 2-3 mm. long.

Type locality.—Crevices of rocks, mountains of Lake Co.

Material studied.—CALIFORNIA: Trinity Co., without locality, *Rattan* in 1883 (G), between the forks of Trinity River, *Rattan* in 1883 (S); west slope of Black Butte, Mendocino Co., *Rattan* in 1884 (G,S); Glenn Co., 9 miles east of Alder Springs, *Heller* in 1914 (S); between Mud Flat and Bennett Springs, *Heller* 11551 (S); Lake Co., mountains, crevices of high rocks, *Greene* in 1874, type collection (G); near Blue Lakes *Tracy* 1677 (S), Mt. Sanhedrin, *Eastwood* 12889 (P); Mt. Hull, *Hall* 9531 (P); Geysers, Sonoma Co., *Bolander* 3968 (G).

The four conspicuous lateral and transverse callous crests of the upper lip which extend into the throat characterize this species. Pedicel length is so variable that *greenei* may be placed in either the sessile group or the group with pediceled flowers.

7. *COLLINSIA FRANCISCANA* Bioletti, Erythea 1:17. 1893; Jepson

Man. Calif., 903. 1925; *C. sparsiflora* var. *franciscana* Jepson Fl. W. Middle Calif., 399. 1901.

Plant diffusely branched, "erect or reclining on neighboring plants," glabrous below to glandular-pubescent and often viscid above, 20–60 cm. tall; leaves deltoid-ovate to lance-ovate, acute, serrate to serrulate, scabrous-margined, glabrous to sparsely pubescent, 1–5 cm. long, 0.5–3 cm. wide, becoming gradually reduced in the inflorescence to small linear bracts in uppermost whorls; lower leaf petioles 1–2 cm. long; pedicels glandular-pubescent, 0.2–4 cm. long; upper flowers in several-flowered whorls; lower flowers long-pedicled and 1–3 at a node; calyx viscid-pubescent, submembranous, 5–11 mm. long; calyx lobes broadly lanceolate, acute, 2–5 mm. long, 1–3 mm. wide; corolla 12–18 mm. long; corolla tube 5–6 mm. long, 4–5 mm. wide; dorsal saccate region shallow, ca. 1 mm. deep, bearded; upper lip white, purple-spotted at base, 6–10 mm. long; upper lobes ovate-truncate, emarginate, 4–6 mm. wide; sinus 2–3.5 mm. deep; lower lip bluish-purple, sparsely bearded without, 9–13 mm. long; keel 3–4 mm. deep, 1–2 mm. shorter than lateral lobes; lateral lobes obovate, emarginate, 4–7.5 mm. wide; upper pair of filaments bearded, clubbed at base or with appendage ca. 0.5 mm. long; gland often on a very short, bearded base; stigma not lobed; capsule 4–7 mm. long; seeds 8 or more, irregularly round-oblong, flattened when immature but becoming thickened, rugose, 1.5 mm. long.

Type locality.—San Francisco peninsula.

Specimens studied.—CALIFORNIA: San Francisco Co., *Michener & Bioletti* in 1893, probably type material (P,S); Sutro Woods, *Walker* 1067 (P); Mission Hills, *Bolander* 148 (G); San Mateo Co., San Bruno Hills, *Heller* 8463 (S); between San Mateo and Half Moon Bay, *Heller* 8580 (S); Crystal Springs Lake, *Baker* 647 (P); Santa Clara Co., Stanford University, *Elmer* 5050 (S,P); Serpentine Hills near Edenvale, *Ferris* 806 (S); Pacific Grove, Monterey Co., *Elmer* 4686 (P,S).

C. franciscana has been confused with *bicolor* and *bartsiaeifolia*, but can be distinguished from both by the elongate pedicels. The general appearance is that of *bicolor*, but the stamen appendages are merely rudimentary. The leaves are thin, and triangular as compared with the thickened lance-oblong leaves of *bartsiaeifolia*.

8. *COLLINSIA Verna* Nutt., Jour. Acad. Philad. 1:190, t. 9.

1817; Benth. in DC. Prodr. 10:318. 1846; Gray Syn. Fl. N. Am. 2:256. 1878; *C. bicolor* Raf., Cincinnati Lit. Gaz. (for *Neophyton* 2), 84. 1824; *C. bicolor* var. *latifolia* Raf., *l.c.*; *C. bicolor* var. *cordata* Raf., *l.c.*; *C. bicolor* var. *rotundifolia* Raf., *l.c.*; *C. bicolor* var. *verticillata* Raf., *l.c.*; *C. bicolor* var. *integrifolia* Raf., *l.c.*; *C. bicolor* var. *angustifolia* Raf., *l.c.*; *C. alba* Raf., *l.c.*

Stems erect, branching or simple, glabrous below to puberulent above; leaves ovate to deltoid-lanceolate, obtuse to acute, often cordate and clasping at base, crenulate to entire, scabrous, 1-6 cm. long, 0.5-2 cm. wide, becoming gradually reduced to small linear bracts in uppermost whorls of inflorescence; basal leaves usually shorter and broader than cauline leaves, with petioles 1-3 cm. long; flowers 4-6 at a node above, 1-3 below; pedicels nearly erect, puberulent, 0.5-3 cm. long; calyx somewhat pale and submembranous at base, puberulent-glabrate, ciliate-pubescent, 5-8 mm. long; calyx lobes lanceolate, acute, 4-5 mm. long, 1-2 mm. wide; corolla declined, 8-14 mm. long, upper lip white or lavender-tinged, lower lip bright blue; corolla tube ca. 4 mm. long, 2-3 mm. wide; dorsal gibbous region shallow, 1 mm. deep, sparsely short-bearded near base; upper lip almost as long as or 2-3 mm. shorter than lower lip, 6-8 mm. long, 2-3 mm. wide, with region below sinus yellowish and spotted with saffron, sinus 4-5 mm. deep; upper lobes obovate, emarginate, 2-4 mm. wide; lower lip 7-10 mm. long; keel about 3 mm. deep, sparsely bearded without, 1.5-3 mm. shorter than lateral lobes; lateral lobes obovate, emarginate, 3-6 mm. wide; upper filaments bearded for half their length and at base, lower filaments beardless or occasionally sparsely bearded near base; anther sacs yellow; stigma not lobed; capsule 4-5 mm. long, exceeded by calyx lobes; seeds 4 or less, round-oblong, somewhat flattened when immature, becoming thickened, rugose-reticulate, 1.5-2.5 mm. long.

Type locality.—near Gallipolis, Ohio.

Material studied.—NEW YORK: Buffalo, in 1887, collector not known (G). PENNSYLVANIA: Allegheny Co., *Knife* in 1871 (G). KENTUCKY: Lexington, *Short* in 1856 (G). OHIO: Erie Co., *Moseley* in 1903 (G); Licking Co., *H. L. Jones* in 1891 (G); Portage Co., *Webb* in 1923 (G); Black River, *Cook* (P). MICHIGAN: Olivet, *Denslow* in 1875 (G); Agricultural College, *Baker* in 1890 (P); Hubbardston, *Smith* in 1876 (S), *Wheeler* in 1873 (P). INDIANA: Crawfordsville, *Evans* (S); Hanover, *Young* in 1875 (P); Bluffton, Wells Co., *Deam* in

1907 (G,P). ILLINOIS: Somer, Champaign Co., *Pease* in 1909 (G); Chicago, *Hitchings* in 1890 (G); Decatur, *Gleason* in 1897 (G); Bloomington, *Robinson* in 1884 (G); Makanda, *Baker* in 1900 (P). IOWA: Jefferson Co., *Fitzpatrick* in 1896 (G). MISSOURI: Jefferson Co., *Eggert* in 1887 (G); Cass Co., *Broadhead* (S); Vale, *Bush* 4931 (G). LOUISIANA: without locality, *ex Herb. George Thurber* (G).

The main distinguishing characters for this species as compared with *C. violacea* are the size and number of seeds. The upper lip ordinarily almost equals the lower in length, but this is variable, and the upper lip becomes quite noticeably shorter in some specimens, thus approaching that in *violacea*. Then, too, the upper lip is not consistently white, often becoming tinged with lavender as in *violacea*. The leaves are quite variable in size and length, but are rather broader than in *C. violacea*. The corolla lobes are not as deeply notched, although there are some specimens that would rather easily fit in as intermediates between the two species in this respect. Taking typical material of the two, however, they are different in the above respects, and may well be termed separate species, although they are closely allied.

RAFINESQUE took up for *verna* the name *C. bicolor* (Cincinnati Lit. Gaz. 84. 1824), claiming that the plant had been given this specific name as early as 1810 by MILLER and MUHLENBERG under the genus *Herpestis*. Since no evidence has been found of the publication of the name *bicolor* previous to that of *verna*, the name *bicolor* of RAFINESQUE is here considered a synonym.

9. *COLLINSIA VIOLACEA* Nutt., Trans. Am. Phil. Soc. 5:179. 1837; Benth. in DC. Prodr. 10:318. 1846; Gray Syn. Fl. No. Am. 2:256. 1878.

Plants erect, branched or simple, puberulent, 10-35 cm. tall; leaves thickened, oblong to lance-oblong, obtuse to acute, serrulate to entire, scabrous, pubescent, 1-4 cm. long, 0.2-1.5 cm. wide; caulin leaves gradually reduced to small linear bracts in upper inflorescence; basal leaves often ovate, with petioles as long as leaves; flowers 4-6 at a node above, 1-3 below; pedicels nearly erect, puberulent, 0.5-2 cm. long, shorter ones in upper whorls; calyx purplish below, not conspicuously submembranous, pubescent, 5-8 mm. long; calyx lobes lanceolate, acute to obtuse, 3-5 mm. long, 1-1.5 mm. wide; corolla declined, bright violet, upper lip paler,

10-15 mm. long; corolla tube 4-5 mm. long, 2-3 mm. wide, bearded below the lower filaments and around gland; dorsal gibbous region shallow, ca. 1 mm. deep; upper lip 4-6 mm. long, ca. 2 mm. shorter than lower lip, with crested region "marked with reniform yellowish fulvous spot"; sinus 2-3 mm. deep; upper lobes ca. 3 mm. wide, obovate, obcordate, notch approaching 1 mm. in depth; lower lip 6-9 mm. long; keel ca. 1 mm. shorter than lateral lobes; lateral lobes 5-6 mm. wide, obovate, obcordate, notch 1 mm. deep; upper filaments bearded for half their length; stigma rather large, not definitely lobed; capsule 4-5 mm. long, exceeded by calyx lobes; seeds flattened when immature, becoming round-oblong and thickened, rugose-reticulate, ca. 1-1.5 mm. long, 6-12 in number.

Type locality.—On the hills and upland woods of the Arkansas and Red rivers.

Specimens studied.—MISSOURI: Carthage, Palmer in 1903 (G); Swope Park, Bush in 1923 (P). KANSAS: Cherokee Co., Hitchcock 979 (G). ARKANSAS: without locality, Harvey in 1880 (G), Pitcher in 1877 (G); Fayetteville, Harvey in 1879 (G). OKLAHOMA: near Crusher Spur, Murray Co., Stevens in 1913 (G,S); Eufaula, Brainerd in 1908 (G). TEXAS: Elmo, Reverchon 3935 (G).

The seeds of this species are smaller and more numerous than in *verna*. Its range somewhat overlaps that of *verna*, but the two species are definite in seed characters, and can usually be distinguished also by several gross corolla characters.

10. *COLLINSIA SPARSIFLORA* Fisch. & Mey., Ind. Sem. Hort. Petrop. 2:33. 1836.

Stems slender, branched near base or simple, often reddish, glabrous or slightly puberulent, 7-40 cm. tall; leaves glabrous to sparsely puberulent, serrulate to entire, scabrous-margined, linear to oblong, narrowly obtuse, 8-20 mm. long, 1-5 mm. wide, gradually transitional to small linear bracts in upper inflorescence; basal leaves broader and shorter with petioles 5-10 mm. long; flowers 1-2 (rarely 3) at a node, on slender pedicels 5-25 mm. long; calyx submembranous and reddish at base, glabrate, setulose-margined, 5-9 mm. long, from one-third as long as to fully as long as the corolla; calyx lobes ovate or deltoid-lanceolate to linear, acute, 2-5 mm. long, 0.5-2 mm. wide; corolla strongly declined, dorsal side of tube forming almost a right angle with pedicel, or erect and in a straight line with

the pedicel, pale lavender to purple, 5–15 mm. long; corolla tube 3–9 mm. long, 2–7 mm. wide; dorsal region of tube strongly gibbous (and 1–2 mm. deep) to straight, usually sparsely bearded within; upper lip purple-dotted above the callous crests, 2–9 mm. long, sinus 1–3 mm. deep; upper lobes truncate, retuse or emarginate in large-flowered forms, oblong and entire in small-flowered forms, 1–5 mm. wide; lower lip 3–11 mm. long; keel 1–4 mm. deep, usually at least 1 mm. shorter than the lateral lobes, more or less long-bearded without near tip; lateral lobes spatulate, emarginate to entire, 1–5 mm. wide; upper pair of filaments at least sparsely bearded for half their length and often bearded at base; lower filaments glabrous or occasionally bearded near base; stigma somewhat 2-lobed; capsule 3–5 mm. long, more or less exceeded by calyx lobes in fruit; seeds 4–8, round-oblong, somewhat flattened, often acutely wing-margined, rugulose, 2–2.5 mm. long when mature.

Key to varieties

- A. Seeds 5–8; mature capsule 4–5 mm. long.
 - B. Corolla strongly declined; dorsal side of saccate tube forming right angle with pedicel; calyx ca. $\frac{1}{3}$ ($\frac{1}{2}$) the corolla length, lobes subulate, not concealing and scarcely exceeding the saccate tube.
 - C. Corolla over 12 mm. long .. 10a. *C. sparsiflora* var. *arvensis*
 - C. Corolla 9–12 mm. long 10b. *C. sparsiflora* var. *typica*
 - B. Corolla not strongly declined; dorsal side of tube gibbous or in straight line with pedicel; calyx from half as long as to fully as long as corolla, lobes lanceolate to linear, well exceeding and concealing the gibbous region of tube.
 - C. Corolla 9–13 mm. long; calyx 7–12 mm. long, cleft at least $\frac{2}{3}$ of its length; stems simple or branched, erect-appearing. Vicinity of Columbia River south to Butte and Lake counties, California 10c. *C. sparsiflora* var. *brucae*
 - C. Corolla 6–9 (–10) mm. long; calyx 4–7 mm. long, cleft $\frac{1}{2}$ to $\frac{2}{3}$ its length; stems divaricately branched. Central California 10d. *C. sparsiflora* var. *solitaria*
- A. Seeds 4; mature capsule 3–3.5 mm. long; corolla ca. 5 mm. long; bearding on filaments often sparse. Sierran and north-central

California..... 10e. *C. sparsiflora* var. *collina*
10a. *C. SPARSIFLORA* var. *ARVENSIS* (Greene) Jepson, Fl. W.
Middle Calif., 398. 1901; Man. Calif., 904. 1925; *C. arvensis* Greene,
Pittonia 2:232. 1892.

Plants occasionally 35 cm. tall; corolla 12-20 mm. long.

Type locality.—Knight's Valley, Sonoma Co., and adjacent Lake and Marin Cos.

Specimens seen.—CALIFORNIA: Mendocino Co., *Purdy* in 1882 (S); Ukiah, *Bolander* 4642 (G); Lake Co., Lower Lake, *Bowman* 109 (S); Clear Lake, *Pearson* 6584 (P); Napa Co., Calistoga, *Baker* 1984 (P); Napa Valley, *Thurber* 502 (G), *Greene* 87 (G); Sonoma Co., Knight's Valley, *Eastwood* in 1900 (G); Kenwood, *Michener & Bioletti*, in 1893 (P,S); hills southeast of Santa Rosa, *Heller & Brown* 5117 (G,P,S); Healdsburg, *Rattan* in 1880 (S); Fort Ross, *Heller* 6601 (P,S); Marin Co., Martinez, *Rattan* in 1880 (S).

This variety is easily distinguished by the conspicuously large size of the corolla as compared with others of the group.

10b. *C. SPARSIFLORA* var. *typica*, nom. nov.

C. sparsiflora Fisch. & Mey., Ind. Sem. Hort. Petrop. 2:33. 1836; Gray Syn. Fl. No. Am. 2:256. 1878; Jepson Man. Calif., 904. 1925; *C. parviflora* var. *sparsiflora* Benth. in DC. Prodr. 10:319. 1846; Plantae Hartw., 328. 1839-57.

Calyx one-third (to one-half) the corolla length, lobes subulate, not concealing and scarcely exceeding the saccate base of corolla tube; corolla 9-12 mm. long, strongly declined, dorsal side of tube forming a right angle with pedicels.

Type locality.—Vicinity of Fort Ross.

Material studied.—Without locality, *Fremont* 234 (G), "Ross," Herb. Acad. Petrop., without doubt from Fort Ross, type material (G). CALIFORNIA: Ukiah to Largo, *Abrams* 7001 (S); Sonoma Co., Healdsburg, *Rattan* in 1880 (S); Bodega Bay, *Heller & Brown* 5268 (P,S); Petaluma, *Congdon* 76 (G), *Smith* 1380 (S); Santa Rosa, *Bolander* 3810 (G); Santa Rosa to Calistoga, *Chandler* 7549 (P); near Vallejo, Solano Co., *Greene* 63 (G); Martinez, Contra Costa Co., *Rattan* in 1880 (S).

Variation has its extreme representation in the *sparsiflora* group. The size of the corolla, shape of the tube, and characters of the corolla lobes are so variable that there is ample reason for several varieties, yet because of numerous intergradations none of these seems distinct enough for specific rank. In *sparsiflora* var. *typica* we find a comparatively small portion of the whole group repre-

sented. Study of the type brings to light the fact that much of the material formerly classed as *sparsiflora* is worthy of varietal rank. The strongly declined corolla with saccate tube, and the length of the flower separate this variety from the others.

The following specimens may be cited as representing intergradations between *typica* and *bruceae*.—CALIFORNIA: Mt. St. Helena, *Eastwood* 11751 (P); Mt. Sanhedrin, *Eastwood* 12799 (P); Mt. Tamalpais, Marin Co., *Grant* 926 (S), *Abrams* 8070 (S); Contra Costa Co., Antioch, *Davy* 961 (S); Byron, *Bioletti* in 1892 (S); Solano Co., Vacaville, *Platt* in 1898 (P); Elmira, *Rattan* in 1878 (S).

10c. *C. SPARSIFLORA* var. *bruceae* (Jones), comb. nov.—*C. bruceae* Jones, Contr. West. Bot. 12:69. 1908.

Stems simple or branched, appearing erect; calyx from half as long as to fully as long as corolla, cleft one-half to two-thirds its length, lobes lanceolate to linear, well exceeding and concealing the gibbous region of the corolla tube; corolla 9–13 mm. long, not strongly declined, often in a straight line with the pedicel, and appearing tubular; corolla lobes not conspicuous.

Type locality.—Not given, but without doubt Little Chico, Butte Co.

Specimens studied.—WASHINGTON: Klickitat Co., near Bingen, *Suksdorf* 2772 (S); White Salmon, *Suksdorf* 298 (G). OREGON: Rogue River Valley, *Howell* 653 (G); The Dalles, *Howell* in 1882 (S), *Sheldon* 10214 (P,S), *Peck* 3879 (G). CALIFORNIA: Siskiyou Co., near Yreka, *Greene* 730 (G); Shasta Valley, *Buller* 574 (P); Buck Mt., Humboldt Co., *Tracy* 4685 (P); Butte Co., Little Chico, *Bruce* 2063, without doubt type material (G,P,S); Chico, *Bidwell* in 1878 (G), *Heller* 11190 (S); near Clear Creek, *Brown* 161 (S); Sulphur Banks, Lake Co., *Bowman* 162 (S).

This variety seems to be a distinct segregate from *typica*, with a few intergrades from intermediate localities. The long calyx, quite concealing a goodly portion of the corolla, the slightly gibbous corolla tube, and the tubular appearance of the flower separate it from *typica*, while the size of the corolla is the main distinction between *bruceae* and *solitaria*.

10d. *C. SPARSIFLORA* var. *solitaria* (Kellogg), comb. nov.—*C. solitaria* Kellogg, Proc. Cal. Acad. 2:110. 1863; *C. divaricata* Kellogg, l.c. 3:36. 1863.

Plant divaricately branching; calyx 4–7 mm. long, cleft half to two-thirds the corolla length; corolla 6–9 mm. long, tube gibbous or straight.

Type locality.—Vicinity of Oakland.

Material studied.—CALIFORNIA: Mokelumne Hill, Calaveras Co., *Blaisdell* (S); Table Mt. above Rawhide, Tuolumne Co., *Ferris* 1482 (S); Marin Co., *Brewer* 929 (G); Saucilito Hills, *Kellogg* 662, type of *divaricata* (G); San Francisco, *Eastwood* in 1895 (G); Mission Hills, *Rattan* in 1881 (S); San Mateo Co., Lake San Andreas, *Abrams* 2322 (P,S); Crystal Springs Lake, *Baker* 444 (P); Santa Clara Co., Mt. Hamilton, *Heller* 8539 (S), *Elmer* 4718 (S), *Baker* 641 (P); Stanford University, *Atkinson* in 1900 (S); near Frenchman's Lake, *Dudley* in 1893 (S); south of Gilroy Hot Springs, *Dudley* in 1910 (S); Monterey Co., between Goold Creek and Burnett Creek, *Dudley* in 1901 (S); near Jones Ranch, Santa Lucia Mts., *Ferris* 1834 (P,S), Santa Lucia Mts., *Plaskett* 94 (G); Fremont's Peak, San Juan, San Benito Co., *Elmer* 5048 (S); Atascadero Creek, San Luis Obispo Co., *Munz* 9221 (P); S.W. of Coalinga, Fresno Co., *Munz* 9176 (P).

Although the calyx length is somewhat variable in this variety, it is at least half the length of the corolla, except in specimens from Lake San Andreas (*Abrams*) in which it is one-third as long as the tubular corolla. Size and shape of corolla are variable, but both the corolla length and the divaricately branching habit make *solitaria* distinct.

10e. *C. SPARSIFLORA* var. *collina* (Jepson), comb. nov.—*C. parviflora* var. *collina* Jepson Man. Calif., 904. 1925.

Plant slender, erect or divaricately branched, leaves ca. 1 cm. long; calyx almost equaling corolla length, lobes lance-linear; corolla ca. 5 mm. long, tube gibbous or straight; bearding on upper filaments often sparse; mature capsule 3-3.5 mm. long; seeds 4.

Type locality.—Penn Valley, foothills of Nevada Co.

Specimens studied.—CALIFORNIA: fields of Butte Co., *Bruce* 2062 in part (P); Lakeport, *Bentley* in 1917 (S); Sacramento, *Brown* in 1903 (S), *Hannibal* in 1918 (S); Live Oak, San Joaquin Co., *Rattan* in 1883 (S); El Dorado Co., *Rattan* in 1866, in part (S); between Clarksville and Shingle Springs, *Heller* 12289 (S); Kelsey, *Jones* in 1903 (P); Stoney Creek, Amador Co., *Hansen* 1525 (S); Penn Valley, Nevada Co., *Jepson* in 1892, type collection (J).

This slender small-flowered variety is distinguishable by the number of seeds and the slightly smaller capsule. It is to be referred to this species rather than to *parviflora* because of its bearded stamens; its corolla characters, too, place it in this group.

11. *COLLINSIA PARRYI* Gray Syn. Fl. N. Am. 2:257. 1878; Jepson Man. Calif., 904. 1925; *C. cahonis* Jones, Contr. West. Bot. 12:68. 1908.

Plants erect or diffusely branched, retrorse-puberulent, 9-45 cm. tall; leaves lanceolate to lance-linear, obtuse to acute, sessile or with petiole 1-2 mm. long, glabrate, serrate to entire, 5-45 mm. long, 1-12 mm. wide, becoming gradually reduced to small linear bracts in uppermost whorls of inflorescence; lower leaves ovate to oblong, obtuse, with petioles 0.5-2 cm. long; flowers 2-3 (1-5) at a node; pedicels retrorse-puberulent, 0.5-4.5 cm. long, subtended by leaves; calyx retrorse-puberulent, ciliate-pubescent, 4-7 mm. long, at least equaling the length of corolla tube; calyx lobes broadly lanceolate, somewhat obtuse, 2-3 mm. long, ca. 1.5 mm. wide; corolla declined, lavender-blue, 5-10 mm. long; corolla tube paler, 3-5 mm. long, 2-4 mm. wide; dorsal gibbous region shallow, ca. 1 mm. deep, rather long-bearded within; upper lip 3-5 mm. long, its sinus 1-2 mm. deep, subtended by a triangular purple-dotted callous region from which the truncate-emarginate (rarely entire) lobes 1.5-2 mm. wide fold back; lower lip 4-7 mm. long; keel about 1 mm. shorter than the lateral lobes, 1-1.5 mm. deep; lateral lobes broadly spatulate, emarginate (rarely entire) 1.5-3 mm. wide; lateral sac occasionally gland-bearded; upper pair of filaments bearded for half their length (occasionally sparsely so); lower filaments glabrous or rarely sparsely bearded at base; stigma not lobed; capsule 4-5 mm. long, scarcely exceeded by calyx lobes; seeds 8-12, round-oblong, flattened when immature, thickened at least on edges when mature, rugulose, tan, ca. 1 mm. long.

Type locality.—San Bernardino Co., California.

Specimens studied.—CALIFORNIA: without locality, Coulter 597 (G), Parry 221 (G); Santa Monica, Hasse in 1890 (S); Soldier's Home, Los Angeles Co., Hasse in 1890 (G); Newhall, Hasse in 1892 (G), Hasse in 1893 (S); Pasadena, Grant in 1900 (P,S); Verdugo Hills, Abrams 1404 (S); San Gabriel Mts., San Gabriel Canyon, Munz 9432 (P), at foot of canyons north of Claremont, Quibell in 1927 (P); San Antonio Canyon, Peirson in 1920 (P); Lytle Creek Canyon, Munz 8153 (P); Cajon Pass, Jones in 1903, type of *cahonis* (not from Tehachapi, as given in type description) (P); San Bernardino Co., Lemmon 1178 (G), Parry & Lemmon in 1876, type collection (G); San Bernardino Mts., Parish 912 (G,S); Waterman Canyon, Jones in 1926 (P); below Seven Oaks, Newsom in 1926 (P).

This species is distinct enough, but probably a segregate of the *sparsiflora* group. The bearding on the filaments, the numerous

seeds that are thickened when mature, and the limited geographical range separate it from other species with which it might be confused. Specimens from two regions are peculiar. Those from Cajon Pass (*Jones*) and Newhall (*Hasse*) are rather succulent and approach the broadened calyx of *C. callosa*, while those from Soldier's Home (*Hasse*) and Santa Monica (*Hasse*) often have very sparse bearding on the stamens, and the upper and lower lateral lobes of the corolla are entire and not emarginate.

12. *COLLINSIA PARVIFLORA* Dougl. in Lindl., Bot. Reg. 13:t. 1082. 1827; Benth. in DC. Prodr. 10:318. 1846; Gray Syn. Fl. No. Am. 2:256. 1878; Jepson Man. Calif., 904. 1925; *C. minima* Nutt., Jour. Acad. Philad. 7:47. 1834; *C. pauciflora* of Hook. Fl. Bor. Am. 2:94. 1838; *C. tenella* of Piper, Contr. U.S. Nat. Herb. 11:496. 1906.

Stems ascending or erect, branched, glabrate to puberulent, often inconspicuously glandular, 5-40 cm. tall; leaves serrulate to entire, glabrate-puberulent or sparsely glandular, often purplish below, 0.7-5 cm. long, 2-8 mm. wide; oblong to lance-linear, becoming smaller and linear-bracteate in inflorescence and ca. 5 mm. long in uppermost whorls; lower leaves ovate to oblong-ovate with petioles 5-10 mm. long; flowers 2-5 in a whorl above, usually solitary below; pedicels puberulent to glandular-pubescent, 3-15 mm. long; calyx somewhat membranous below, puberulent to glabrate, scabrous-margined, 3-7 mm. long, from half as long as to almost equal the corolla length; calyx lobes subulate to linear-lanceolate, acuminate-acute, ca. 1 mm. wide; corolla declined to almost erect, 4-6 mm. long, tube white to pale blue, lobes a clear blue, often pale, upper lip varying to white; corolla tube half the length of the corolla, throat compressed, making the tube seem two-thirds the corolla length; dorsal gibbous region abrupt but shallow, ca. 0.5 mm. deep, short-bearded to glabrous; upper lip 2-3 mm. long, sinus ca. 1 mm. deep, lobes obovate-spatulate, slightly recurved from crested region; lower lip 2.5-3.5 mm. long; keel 1 mm. deep, ca. 1 mm. shorter than lateral lobes; lateral lobes spatulate, ca. 1 mm. wide; filaments rather stout, glabrous; stigma 2-lobed; capsule 3-4 mm. long, slightly exceeded by calyx tips; seeds normally 4, 1-2 mm. long, round-oblong, thick, smooth, reddish-brown or brown.

Type locality.—Vicinity of Columbia River.

Representative material¹.—CANADA: Vancouver Is., Victoria, *Macoun* 54 (G); Comox, *Macoun* 717 (G); Bald Mt., Cowichan Lake, *Rosendahl* 1833 (G); Selkirks, B.C., *Brown* 293 (G); Field, B.C., *Brown* 347 (G); Banff, Alta., *Moodie* 1237 (G); Lake Winnipeg Valley, *Bourgeau* in 1851 (G). WASHINGTON: Hump-tulips City, *Lamb* in 1897 (S); Button Springs, *Leiberg* 354 (G,P); Conconully, *Jones* in 1911 (P); Pullman, *Duthie* (S); Swauk River, Kittitas Co., *Sharples* 224 (G). OREGON: Multnomah Co., *Howell* 265 (G); Redmond, *Whited* in 1912 (S); 25 miles southeast of Pendleton, *Munz* 9914 (P); Grant's Pass, *Prescott* in 1912 (G). CALIFORNIA: Yreka, *Buller* 1133 (P,S); Little Hot Springs Valley, Modoc Co., *Baker* & *Nutting* in 1894 (S); Kneeland Prairie, Humboldt Co., *Rattan* in 1882 (S), *Tracy* 2658 (S); Prattville, *Heller* & *Kennedy* 8808 (S); Jonesville, Butte Co., *Heller* 12048 (S); Fallen Leaf Lake, El Dorado Co. (S); Emigrant Gap, *Jones* 3293 (P); Reverse Creek, Silver Lake Region, *Peirson* 6136 (P); lower end of Donner Lake, *Heller* 6867 (P,S); Bonita Meadow, Tulare Co., *Hall* & *Babcock* 5186 (S); San Bernardino Mts., Bear Valley, *Parish* 3754 (G), *Munz* 8189 (P), South Fork of Santa Ana River, *Peirson* 3141 (S); San Jacinto Mts., *Hall* 1810 (S); south of Cuyamaca Lake, *Munz* & *McCully* 8124 (P); Laguna Mts., San Diego Co., *Munz* 9673 (P). ARIZONA: Chiricahua Mts., *Lemmon* 3073 (G). NEVADA: Big Creek, Lander Co., *Kennedy* 4048 (S). UTAH: Alta, Wasatch Mts., *Jones* in 1879 (P); Marysville, *Jones* 5384 (P); Red Butte, *Clemens* in 1908 (S); Salt Lake City, *Walson* 769 (G). IDAHO: Deer Lake, Washington Co., *Jones* in 1899 (P); Silver City, Owyhee Co., *Macbride* 929 (P,S); Pocatello, Bannock Co., *Pennell* in 1915 (S); Moscow, Latah Co., *Abrams* 540 (P,S); Coeur d'Alene Lake, *Jones* in 1905 (P); west of Ashton, Fremont Co., *Pennell* 6051 (G). MONTANA: Big Fork, Flathead Lake, *Clemens* in 1908 (G,S), *Jones* 8908 (P); Bozeman, *Scribner* 182 (G), *Moore* in 1901 (P,S); Spanish Creek, Gallatin Co., *Vogel* in 1901 (G). WYOMING: Halleck Canyon, Albany Co., *Nelson* 7430 (P); Newcastle, *Bates* in 1896 (G); 12 miles north of Evanston, Uinta Co., *Pennell* 5911 (G,S); hills east of Afton, Lincoln Co., *Payson* & *Armstrong* 3247 (P). COLORADO: Larimer Co., *Baker* 7932 (P); Boulder, *Vestal* 343 (S); Wolhurst, Douglas Co., *Duthie* & *Clokey* 3864 (G,P,S); Sulphur Springs, Grand Co., *Osterhout* 3233 (G,S); near Golden, *Jones* 250 (P), *Pennell* 5818 (G); Chicken Creek, West La Plata Mts., *Baker*, *Earle*, *Tracy* 820 (P). SOUTH DAKOTA: Elk Canyon, Black Hills, *Rydberg* 913 (G). MICHIGAN: Hancock, *Robbins* 160 (G).

The *C. tenella* of Piper (Contr. U.S. Nat. Herb. 11:496. 1906) is based on *Antirrhinum tenellum* Pursh Fl. 2:421. 1814. PURSH's description is based on a collection made by Lewis on the LEWIS and CLARK Expedition. PURSH states that the plant was found "on the banks of the Missouri" in July. In the Academy of Natural Sci-

¹ Such herbarium sheets have been selected for citation from those studied as will give some indication of range.

ences at Philadelphia there is a specimen of *C. parviflora* collected on April 19, 1806 at Rockford Camp. This plant was discussed by MEEHAN (Proc. Philad. Acad. 50:12-49. 1898) who reported identifications made by ROBINSON and GREENMAN. They considered it as representing *Antirrhinum tenellum* Pursh, and, according to the data with the specimen, from Rockford Camp, at the mouth of Mill Creek, near the Dalles, Ore. DR. PENNELL has kindly examined this specimen for me and writes that it disagrees with PURSH's description in having the stem branched and pubescent, the flowers long-pediced and doubtless small, although they have been lost from the specimen. He suggests that it is very doubtful whether this specimen represents *Antirrhinum tenellum* Pursh, although he has no other plant to suggest. Because of this uncertainty with regard to the species name *tenella*, it seems better to maintain *parviflora*, for which species there is authentic material, even though the former name has been used in many recent manuals (Coulter & Nelson, Rocky Mt. Manual, 439. 1909; Britton & Brown, Fl. No. Sts. & Can. 3:189. 1913, ed. 2; Wooton & Standley, Contr. U.S. Nat. Herb. 19:579. 1915; Tidestrom, Contr. U.S. Nat. Herb. 25:485. 1925).

With such a large geographical range, since *parviflora* is by far the most widespread of all the species of *Collinsia*, one would expect that there would be several differences found worthy of varietal rank. The most noteworthy variations are as follows: (1) the more slender habit of the Canadian plants, (2) the somewhat thickened leaves of plants from the southern part of Idaho and Utah, and (3) broad-leaved forms from Montana. But such indefinite and minor variations seem unworthy of nomenclatural recognition. This species is distinct from *grandiflora* var. *pusilla*, which it resembles, in corolla length and in the possession of an almost erect and gibbous corolla tube in contrast to a declined and saccate tube. In studying these two closely allied species, one finds a continuous series of intergradation from the largest-flowered *grandiflora* var. *typica* through *grandiflora* var. *pusilla*, to the smallest-flowered *parviflora*, and yet the two extremes differ greatly. The saccate tube of *grandiflora* gradually gives way to the gibbous tube of *parviflora* or vice versa, and so with the other changing characteristics. Some material

from southeastern Washington and adjacent parts of Idaho is rather hard to place, for the flowers have the characteristics of rather small-flowered *grandiflora* var. *pusilla*, but occur in regions where *parviflora* also is found, and would ordinarily be taken for *parviflora* (Pullman, Wash., Elmer 162.-P). On careful examination, however, it is found that the corollas of these specimens are declined and saccate. After considering also the length of the corolla I have placed them in *grandiflora* var. *pusilla*. I have included some doubtful material collected by Lemmon (3073) from the Chiricahua Mts. in Arizona. It is not at all typical, for the corolla throat is open, the upper lip not at all reflexed, the tube slender and not gibbous, and the plant quite conspicuously glandular. Additional material from the same locality is desirable. The only other Arizona collection seen (Prescott, Palmer 635, G) is quite typical.

13. *COLLINSIA GRANDIFLORA* Dougl. in Lindl., Bot. Reg. 14: t. 1107. 1827; Hooker Fl. Bor. Am. 2:94. 1838.

Stems erect, simple, branching, or rarely somewhat decumbent, glandular-pubescent above to glabrate below, 5-40 cm. tall; leaves oblong to lanceolate, becoming linear in upper whorls, obtuse to acute, sessile or occasionally very slightly petiolate, glabrate, serrulate to entire or entire-revolute, scabrous-margined, 1-4 cm. long, 0.3-1.3 cm. wide becoming smaller and bractiform in inflorescence; lower leaves oblong to spatulate, obtuse, with petioles 5-10 mm. long; flowers 3-7 at a node, rarely solitary; pedicels puberulent, inconspicuously glandular, 0.3-2 cm. long; calyx membranous below, glabrate-puberulent, scabrous-margined, 4-7 mm. long; calyx lobes subulate, acuminate, 2-5 mm. long, 1-1.5 mm. wide; corolla strongly declined, 7-15 mm. long, forming a right angle with the pedicel, upper lip white to purple, lower lip deep blue-violet, lips reflexed about half the corolla length; corolla tube 3-5 mm. long, 2-4 mm. wide; dorsal saccate region deep at base, somewhat declined toward mouth of tube, sparsely bearded or glabrous, 1.5-3 mm. deep; upper lip 3-6 mm. long, sinus 1-4 mm. deep; upper lobes recurved from narrow callous ridge of upper lip, obovate, retuse-entire, 2-4 mm. wide; lower lip 4-9 mm. long; keel 1.5-3 mm. deep, ca. 1 mm. shorter than lateral lobes; lateral lobes obovate, emarginate-retuse, occasionally entire, 2-5.5 mm. wide; filaments rather stout, glabrous,

the upper pair often bearded at lowest attachment point; stigma two-lobed, but not always plainly so; capsule 4-5 mm. long, exceeded only by calyx tips; seeds normally 4, thickened, round-oblong, smooth, reddish-brown when mature.

Key to varieties

A. Corolla 7-9 mm. long; lobes reflexed $\frac{1}{2}$ - $\frac{1}{3}$ the corolla length
 13a. *C. grandiflora* var. *pusilla*
 A. Corolla over 10 mm. long, broad lobes reflexed about $\frac{1}{2}$ the corolla length.....13b. *C. grandiflora* var. *typica*
 13a. *C. GRANDIFLORA* var. *PUSILLA* Gray Syn. Fl. No. Am. 2:256. 1878; Jepson Man. Calif., 904. 1925; *C. pusilla* Howell Fl. N.W. Amer. 1:506. 1901; *C. multiflora* Howell Fl. N.W. Amer. 1:506. 1901; *C. parviflora* var. *minima* (Nutt.) Jones, Contr. West. Bot. 12:69. 1908.

Corolla 7-9 mm. long, tube saccate at base; corolla lobes rather broad, reflexed one-half to one-third the total corolla length.

Type locality.—"Plumas Co., California."

Specimens studied.—BRITISH COLUMBIA: Vancouver Is., Victoria, *Macoun* 718, 719, 720 (G), Nanaimo, *Fowler* 8 (G), Oak Bay, Victoria, *Pineo* in 1901 (P). WASHINGTON: Puget Sound, *Cooper* (G); Olympic Mts., Clallam Co., *Elmer* 2586 (P,S); Sequim, *Grant* in 1916 (S), *Grant* 234 (G); Langley, *Grant* in 1922 (G); Pullman, *Elmer* 162, type of *parviflora* var. *minima* (P 14470, 45049); Hangman Creek, Spokane Co., *Sandberg* & *Leiberg* in 1893 (G,P). OREGON: Willamette Heights, Portland, *Sheldon* 10308 (G,P,S); Orcas Is., *Lyall* in 1858 (G); west of Salem, *Nelson* 2038 (G); Roseburg, *Howell* in 1881 (S); The Dalles, *Lunell* in 1903 (G,S); near Lexington, Morrow Co., *Leiberg* 17 (G); near Harper Ranch, Mathew Co., *Leiberg* 2085 (G). CALIFORNIA: without locality, *Rattan* in 1883 (S); Plumas Co., *Mrs. Austin* without data, type collection (G). IDAHO: Hatwai Creek, Nez Perces Co., *Sandberg*, *McDougal*, *Heller* 38 (G,S).

This variety is characterized by the rather small size of the corolla and the narrower and shorter corolla lobes.

13b. *C. GRANDIFLORA* var. *typica*, nom. nov.—*C. grandiflora* Dougl. in Lindl., Bot. Reg. 14:t. 1107. 1827; Hooker Fl. Bor. Am. 2:94. 1838; Benth. in DC. Prodr. 10:318. 1846; Gray Syn. Fl. No. Am. 2:256. 1878; Jepson Man. Calif., 904. 1925; *C. Diehlii* Jones, Contr. West. Bot. 12:68. 1908.

Corolla 10-15 mm. long, the tube saccate at base; corolla lobes broad, retuse, reflexed half the total corolla length.

Type locality.—"Dry gravelly banks of the Columbia."

Material studied.—BRITISH COLUMBIA: Vancouver Is., *Wood* in 1859-60 (G). WASHINGTON: Mt. Hamilton, *Gorman* 4588 (S); Steilacoom, *Cooper* (G); near Lower Cascades, *Suksdorf* in 1886 (G); Bingen, Klickitat Co., *Suksdorf* 184 (S). OREGON: Portland, *Jones* in 1902 (P); Oregon City, *I. E. Diehl* in 1902, type of *Diehlia* (P); Salem, *Nelson* 1065, 1177 (G); Forest Grove, *Henderson* in 1883 (G); Grant's Pass, *Henderson* 1379 (G); Multnomah Co., *Howell* 263 (G), La Turelle Falls, *Sheldon* 11910 in 1903 (S). CALIFORNIA: Quartz Valley, Siskiyou Co., *Butler* 1226 (P); Redwood Belt, Humboldt Co., *Chandler* 1251 (S); Mong Valley, Mendocino Co., *Bolander* 4675 (G); Sierra Valley, Big Meadows, *Lemmon* in 1879 (G).

This variety is quite distinct with its large flowers and broad reflexed lobes. There is great variation in the size of plants but this has little significance taxonomically.

14. *COLLINSIA RATTANII* Gray, Proc. Am. Acad. 15:50. 1880.

Plant 10-40 cm. tall; stems much branched, or occasionally simple, puberulent-glabrate below, glandular-pubescent above, giving a dull hue to plant; leaves thickened, gray-green, often purplish below, linear-elongate, entire and revolute to serrulate, obtuse, glabrous, 1-6 cm. long, 2-6 mm. wide, becoming gradually reduced in inflorescence to small linear bracts 2-5 mm. long in uppermost whorls; basal leaves much shorter, ovate to oblong, with petioles 5-10 mm. long; flowers 1-4 at a node; pedicels conspicuously glandular-pubescent, 3-10 mm. long; calyx one-third to two-thirds the length of corolla, glandular-pubescent, cleft half its length, 2.5-5 mm. long; calyx lobes deltoid-lanceolate, acute, 1-2.5 mm. long; corolla gibbously declined to erect, light blue to violet, 5-14 mm. long; corolla tube 2-5 mm. long, 1.5-4 mm. wide; dorsal saccate region 1-2.5 mm. deep, sparsely short-bearded within; upper lip 2.5-11 mm. long, one-third to two-thirds of its length reflexed; upper lobes ovate-oblong, retuse-entire, sinus 1-3 mm. deep; lower lip 2-11.5 mm. long; keel as long as or ca. 1 mm. shorter than lateral lobes, 1-3 mm. deep, often sparsely bearded without; lateral lobes ovate to spatulate, retuse-entire, 1-5.5 mm. wide; lateral saccate region bearded; upper filaments bearded at basal attachment, often with a rudimentary bearded appendage ca. 0.3 mm. long; stigma not lobed; capsule 4-5 mm. long, equaled by calyx lobes; seeds somewhat flattened, slightly margined, oblong, 1.5-2 mm. long, 2-6 in number.

Key to varieties

A. Corolla less than 7 mm. long, appearing tubular, lobes inconspicuous; corolla lips no longer than tube

14a. *C. rattanii* var. *typica*

A. Corolla over 7 mm. long, lobes reflexed, conspicuous; corolla lips longer than to twice as long as the tube

14b. *C. rattanii* var. *linearis*

14a. *C. RATTANII* var. *typica*, nom. nov.—*C. rattanii* Gray, Proc. Am. Acad. 15:50. 1880; Syn. Fl. No. Am. 2:439 (supplement). 1886; *C. glandulosa* Howell Fl. N.W. Amer. 1:506. 1901; *C. torreyi* var. *rattanii* Jepson Man. Calif., 905. 1925.

Corolla less than 7 mm. long, tubular; corolla lobes inconspicuous, usually not as long as the tube.

Type locality.—Open hillsides south of Trinity River, California.

Specimens studied.—WASHINGTON: West Klickitat Co., *Suksdorf* in 1886 (G); White Salmon, *Suksdorf* 301 (G). OREGON: Currant Creek, *Howell* 507 (G); The Dalles, *Howell* in 1882 (S); Cold Camp, John Day Valley, (photograph) *Howell* in 1885, type of *glandulosa* (G); state line between California and Oregon, *Diehl* 129 (P). CALIFORNIA: Siskiyou Co., Quartz Valley, *Butler* 609 (P), Klamath River, *Butler* 1188 (P,S); Black Butte, *Rattan* in 1884 (S); south of Trinity River, *Rattan* in 1879, type collection (G); Trinity and Humboldt Cos., *Rattan* in 1883 (G); near Bennett Spring, Glenn Co., *Heller* 11949 (S); Mt. Sanhedrin, Lake Co., *Eastwood* in 1925 (P), *Rattan* in 1884 (G,S).

There is much variation in the vegetative forms of this species, but in the corolla is found a definite intergradation with respect to size and shape. This corolla series is so marked that *linearis* is now recognized as only a variety of *rattanii*. Indeed, GRAY seems to have had difficulty too, for a specimen collected by GREENE, 742a, in 1876 (G), is cited by GRAY as *rattanii* in the original description of that species. The same specimen is cited as *linearis* in Syn. Fl. N. America, supplement, 2:439. 1886. The specimen is decidedly on the borderline between the two varieties, but because of the size of the corolla, it is here placed with *linearis*. *Typica* is characterized by the small, tubular corolla with short, inconspicuous lobes. The *rattanii* group is separated from the *torreyi* group, with which JEPSON confused it, by the presence of bracts in the upper whorls of the inflorescence.

14b. *C. RATTANII* var. *linearis* (Gray), comb. nov.—*C. linearis* Gray, Proc. Am. Acad. 15:50. 1880; Syn. Fl. No. Am. 2:439 (supplement). 1886; *C. torreyi* var. *linearis* Jepson Man. Calif., 905. 1925.

Corolla over 7 mm. long, lobes reflexed and conspicuous; corolla lips longer than twice as long as the tube.

Type locality.—Along Klamath and Trinity rivers, on argillaceous-rocky hills, N.W. California.

Specimens studied.—OREGON: Waldo, *Rattan* in 1879 (G), *Howell* 229 (G,S); Siskiyou Mts., Jackson Co., *Abrams & Benson* 10199 (S); between Grove and Wolf creeks, Josephine Co., *Abrams & Benson* 10433 (S); Grant's Pass, *Heller* 10020 (S), *Dale* in 1913 (S); Gerlinger, Polk Co., *Nelson* 2070 (G). CALIFORNIA: without locality, *Greene* 742a (G); near Yreka, *Greene* 742 (G); Humbug Mt., Siskiyou Co., *Buller* 1262 (P,S), *Buller* 758 (P); Shasta Retreat, *Heller* 7946 (S); south of Trinity River, *Rattan* 21 (G); along Klamath and Trinity rivers, *Rattan* 54, in 1879, type collection (G); Hoopa Valley, Humboldt Co., *Rattan* in 1878 (S), *Chandler* 1294 (S).

This variety has much variation in corolla size in specimens from a given locality, as from Waldo, Oregon (*Howell*; *Rattan*) and Grant's Pass (*Dale*; *Heller*), but the increase in corolla size and the broader and more conspicuous corolla lobes make it distinct from *typica*.

15. *COLLINSIA CHILDII* Parry, in Gray Syn. Fl. No. Am. 2:257. 1878; Jepson Man. Calif., 905. 1925; *C. inconspicua* Congdon, Erythea 7:187. 1900; *C. breviflora* Suksdorf, West. Am. Sci. 12:54. 1901.

Plant erect, much branched, glabrate to puberulent below, glandular-pubescent above, 10–40 cm. tall; leaves serrulate to entire-revolute, scabrous-margined, glabrous, ovate-rotund to oblong or lanceolate, obtuse-acute, 1–5 cm. long, 0.5–1.5 cm. wide; lower leaf petioles not over 1 cm. long; flowers 2–4 at a node, occasionally 1–5; pedicels glandular-puberulent, 0.5–1 (–2) cm. long, the lower subtended by leaves, the upper by oblong-linear bracts less than 1 cm. long; calyx scarious, each lobe with 1–3 darker green veins, glandular-pubescent, 4–7 mm. long; calyx lobes broadly lanceolate, narrowly obtuse to acute, 2–4 mm. long; corolla lavender to white, region below the sinus of upper lip purple-spotted, 6–8 mm. long; corolla tube 3–4 mm. long, 1.5–2.5 mm. wide; dorsal gibbous region shallow,

ca. 0.5 mm. deep, usually with short gland-bearding at the base; upper lip 2-2.5 mm. long, the sinus 1-1.5 mm. deep; upper lobes broadly obtuse, ca. 1.5 mm. wide; lower lip 2-3 mm. long; keel ca. 1 mm. shorter than lateral lobes, 1 mm. deep, often gland-bearded without; lateral lobes spatulate, 1-1.5 mm. wide; lateral sac occasionally sparsely gland-bearded; filaments glabrous; stigma not lobed; capsule 3-4 mm. long, exceeded by calyx lobes; seeds 2, thick, round-oblong, ca. 3 mm. long.

Type locality.—"South-eastern California, in deep woods in the San Bernardino Mts."

Specimens studied.—CALIFORNIA: along Truckee River, Placer Co., Sonne 252 (S); Mariposa Co., Putman Mt., Congdon 77 (G); Hegan Mt., Congdon 61, type of *inconspicua* (G); Tassajara Hot Springs, Monterey Co., Elmer 3362 (S); San Rafael Mts., Ford in 1887 (G); Pine Ridge, Fresno Co., Hall & Chandler 86, type of *breviflora* (S); Greenhorn Range, Kern Co., Hall & Babcock 5028 (S); Tehachapi, Davidson in 1895 (S); San Antonio Mts., Bear Canyon, Peirson 2156 (P); Upper San Sevaine Flats, Johnston 5111 (P); San Bernardino Mts., Parry & Lemmon 298, probably type collection (G), Parish 911 (S); Burnt Mill Ranger Station, Munz 8155 (P); Fredalba, Munz & Williams 2940 (P); Palomar Mts., Hall 1942 (P,S); Parish 4407 (G); Cuyamaca Mts., Brandegee in 1894 (S); Laguna Mts., Mearns & Schoenfeld in 1894, in part (S); Descanso Creek, San Diego Co., Munz & McCully 8098 (P).

Characterized by the broadened, serrulate-entire, thin leaves, and by the calyx, which is membranous at base and exceeds the capsule, *childii* is distinct from all other species.

16. *COLLINSIA CALLOSA* Parish, Erythea 7:96. 1899; Jepson Man. Calif., 905. 1925.

Plant stout, erect, diffusely branching, glabrous below, glandular-pubescent above, 5-25 cm. high; leaves thickened, succulent, glabrous, purplish below, oblong to ovate, obtuse to acute, entire; margins revolute, not over 3 cm. long, 1 cm. wide; gradually reduced to small linear bracts in upper inflorescence; lower leaves sessile or with petioles 1-4 mm. long; pedicels glandular-pubescent, 0.5-1.5 cm. long, 1-3 at a node; calyx glandular-pubescent to glabrate, 5-7 mm. long, becoming conspicuously broad (5 mm.) at base in fruit; calyx lobes broadly deltoid-lanceolate, acute, 2-3 mm. long, ca. 1 mm. wide in flower, becoming ovate-acute, 2-3 mm. wide in fruit; corolla rose-lavender, 6-10 mm. long; corolla tube yellowish, 3-5

mm. long, ca. 2 mm. wide; dorsal saccate region shallow, ca. 1 mm. deep, short-bearded to glabrous within; upper lip 3-4 cm. long, 2-3 mm. wide, its sinus 1.0-1.5 mm. deep, subtended by a yellow-rose-colored purple-spotted region above the callosity; upper lobes round-obtuse (rarely emarginate), 1.0-1.5 mm. wide; lower lip ca. 0.5 mm. longer than upper lip; keel 1 mm. shorter than lateral lobes, ca. 1 mm. deep; lateral lobes narrowly spatulate, obtuse, ca. 1 mm. wide; lateral sac yellowish, calloused, 2-3 mm. long; filaments pale lavender, glabrous, upper pair rarely sparsely bearded and with a rudimentary bearded basal appendage ca. 0.4 mm. long; anther sacs purple; stigma not lobed; capsule 4-6 mm. long, slightly longer than the calyx lobes; seeds 6, oblong, thickened, rugose-reticulate, ca. 2 mm. long.

Type locality.—Swartout Canyon, San Antonio Mts.

Material studied.—CALIFORNIA: Panamint Mts., near Willow Creek, Coville & Funston 750 (G,S); Millspaugh, Argus Mts., Hall & Chandler 7079 (P); Lee District, Nelson Range, Hall & Chandler 7149 (P); foothills south of Bishop, Heller 8290 (S); Mt. Piños, Cuddy's, Dudley & Lamb 4516 (P,S); Seymour Creek, Munz 6961 (P); Tehachapi, Jones in 1903 (P), Water Canyon, Abrams & McGregor 439, in part (S); Lancaster, Davidson in 1895 (S); Mt. Gleason, Acton, Elmer 4178 (S); San Antonio Mts., Hall in 1899, probably type collection (S), Swartout Canyon, Hall 1492 (P,S); Big Rock Creek, Munz 6803 (P); hills bordering Mojave Desert, Pringle in 1882 (G); Crystal Creek, San Bernardino Mts., Jaeger in 1927 (P).

C. callosa has the appearance of a desert form of *childii*, but with the more numerous and smaller seeds, and the thick, succulent leaves and calyces, it is quite distinctive.

17. *COLLINSIA TORREYI* Gray, Proc. Am. Acad. 7:378. 1868.

Stems erect, widely branched, occasionally simple, glandular, very floriferous, 5-20 cm. tall; leaves linear to elliptical, slightly thickened, glabrous, entire to serrulate, 1.5-4.5 cm. long, 0.5-10 mm. wide; lower leaves smaller, spatulate-oblong, with petioles 5-10 mm. long; flowers commonly in 3-6 flowered whorls, occasionally fewer; pedicels glandular, 5-10 mm. long, bracts obsolete or less than 2 mm. long above, to filiform and 5-10 mm. long below; calyx moderately glandular, 2-4 mm. long, cleft for half its length, lobes linear-obtuse, 0.5-1 mm. wide; corolla 4-9 mm. long, gibbously declined, all blue-violet or the upper lip paler or yellowish to white;

corolla tube 2-4 mm. long, 1.5-3 mm. wide, throat broad and open; dorsal gibbous region shallow, less than 1 mm. deep, glabrous or sparsely bearded within near base; upper lip 2-4 mm. long, sinus ca. 1 mm. deep, lobes truncate, 1-2 mm. wide, somewhat reflexed from the callous ridge which has 2 toothlike crests; lower lip 2.5-5 mm. long; keel as long as to ca. 2 mm. shorter than lateral lobes, 1-2 mm. deep; lateral lobes broadly spatulate, obtuse-truncate, 1-2 mm. wide; filaments glabrous or the upper pair bearded at basal attachment, appearing broad; stigma 2-lobed, often inconspicuously so; capsule 2.5-4 mm. long, equaled by calyx lobes; seeds 2, thick, round-oblong, rugulose, 2-2.5 mm. long.

Key to varieties

- A. Flowers 6-9 mm. long.
- B. Keel as long as lateral lobes, or less than 1 mm. shorter.
 - C. Leaves linear, many times as long as wide. Sierra Nevada Mountains. 17a. *C. torreyi* var. *typica*
 - C. Leaves elliptical or lance-ovate, 2-4 times as long as wide. Northern California, southern Oregon
 - 17b. *C. torreyi* var. *latifolia*
 - B. Keel 1-2 mm. shorter than the broad lateral lobes of corolla. Tulare and Fresno counties... 17c. *C. torreyi* var. *brevicarinata*
- A. Flowers less than 6 mm. long; plant 4-20 cm. tall
 - 17d. *C. torreyi* var. *wrightii*

17a. *C. TORREYI* var. *typica*, nom. nov.—*C. torreyi* Gray, Proc. Am. Acad. 7:378. 1868; Syn. Fl. No. Am. 2:257. 1878; Jepson Man. Calif., 905. 1925.

Leaves linear, many times as long as wide; flowers 6-9 mm. long, keel as long as lateral corolla lobes or less than 1 mm. shorter.

Type locality.—Mariposa Big-tree Grove, California.

Material studied.—CALIFORNIA: Grove of large trees (Mariposa Big-tree Grove), *Torrey* in 1865, type collection (G); near Donner Lake, *Torrey* in 1865 (G), *Heller* 6914 (P,S); Emigrant Gap, *Jones* in 1882 (P); Tallac, Lake Tahoe, *Abrams* 7328 (S); Antelope, Amador Co., *Hansen* 4714 (S); Clark's and Yosemite Valley, *Bolander* 4874 (G), vicinity Hog Ranch, *Hall & Babcock* 3393 (P,S), trail from Tioga Road to Yosemite Falls, *Munz* 7521 (P); Hegan Mt., Mariposa Co., *Congdon* in 1894 (S); Huntington Lake, *Grant* 1034 (S); Pine Ridge, Fresno Co., *Hall & Chandler* 61 (S).

C. torreyi differs from the other species of the genus in the absence or minuteness of bracts in the upper whorl of the inflorescence. Within the group are several variations worthy of recognition. In *typica* the color of the corolla varies from blue-violet to forms with a paler or even white upper lip (*Munz*, trail from Tioga Road to Yosemite Falls). It is easily recognized by the large flowers (in contrast with those of *wrightii*) and the long linear leaves.

17b. *C. TORREYI* var. *latifolia*, var. nov.

Folia elliptica aut lanceolato-ovata, ca. 2-4 longiora quam lata; floribus 6-9 mm. longis; labio superiore luteo-albo.

Type, Ashland Butte, southern Oregon, *Cusick* 2893 (type, Pomona College Herbarium no. 42879; cotype at Gray). Other material, OREGON: Ashland Butte, *Howell* in 1887 (S), *Henderson* in 1886 (S); border between Oregon and California, *Henderson* in 1886 (G). CALIFORNIA: Sisson, Siskiyou Co., *Brown* 318 (S); Mts. near Yreka, *Greene* 912 (G); Squaw Creek Ranger Station, *Drew* in 1916 (S); Little Hot Springs, Modoc Co., *M. S. Baker* (P).

The following collection is cited as representing an intergradation between *latifolia* and *typica*.—Dorleska, in Salmon Mts., Trinity Co., Calif., *Hall* 8569 (P,S).

This variety is recognizable by its very broad leaves and its separate geographical range.

17c. *C. TORREYI* var. *brevicarinata*, var. nov.

Flores 6-9 mm. longi; carina quam lobis lateralibus corollae 1-2 mm. breviore; labio superiore luteo-albo.

Type, Hockett's Meadow, Tulare County, California, *Culbertson* 4220 (Pomona College Herbarium no. 14844; cotype, Stanford). Other material, CALIFORNIA: Hockett's Meadow, Dudley 1899 (S); between Mineral King and Farewell Gap, *Hall & Babcock* 5394 (S); Alta, *Grant* in 1902 (S); vicinity of Homer's Nose, Sequoia Nat'l Forest, *Dudley* 1834 (S); Mt. Silliman, Clover Creek, *Dudley* 1467 (S); near Mineral King, *Coville & Funston* 566 (G); Sierra Nevada Mts., *Grant* 199 (S); Fresno Co., Sequoia Mills, *Eastwood* in 1894 (G); Sequoia Horse Corral Meadow, *Dudley* 3167 (S).

Like *wrightii* in leaf characters, and with the corolla appearing like *typica* at a glance, *brevicarinata* is separated by the conspicuously shortened keel of the broad-lobed flowers.

17d. *C. TORREYI* var. *WRIGHTII* (Wats.) Johnston, Plant World 22:115. 1919; Jepson Man. Calif., 905. 1925; *C. wrightii* Wats., Proc. Am. Acad. 24:84. 1889; *C. brachysiphon* Eastw., Bull. Torr.

Bot. Club 32:214. 1905; *C. torreyi* var. *brachysiphon* (Eastw.) Jepson Man. Calif., 905. 1925; *C. monticola* Davidson, Bull. So. Calif. Acad. Sci. 16:13. 1917.

Plants 4-29 cm. tall; bracts of upper whorls of inflorescence obsolete or minute, except when plants are immature and lower bracts are ca. 1 cm. long and appear as the upper ones; flowers 4-6 mm. long.

Type locality.—Greenhorn Mts., Kern Co.

Material studied.—NEVADA: Divide south of Slide Mt., Washoe Co., Heller 10967 (S). CALIFORNIA: Black Butte, Siskiyou Co., *Rattan* in 1884 (S); Lassen Peak, Jones in 1897 (P); above Jonesville, Butte Co., Heller 12028 (S); Soda Springs; Nevada Co., Jones 2394 (G,P); Cisco, Placer Co., Heller 12722 (S), Summit, Heller 9855 (S); El Dorado Co., Mt. Tallac, *McGregor* 190 (S); Half Moon Lake, *McGregor* in 1909 (S); near Camp Echo on Lincoln Highway, Heller 12173 (S); Webber Lake, *Doten* 65 (S); Tuolumne Co., meadows, Ware 2615c, 2630c (G); Yosemite, Gray in 1872 (G); Phillips, Tulare Co., Evans in 1920 (P), Funston's Meadows, Kaweah Peaks, *Dudley* 2189 (S); Tobias Meadow, Kern River Valley, *Dudley* 622 (S); Greenhorn Mts., Kern Co., *Hall & Babcock* 5045 (S), *Palmer* in 1888 (G); 12 miles from Kernville, *Wright* 4, type collection (G,S); Tehachapi Mts., Brook Canyon, *Dudley* 322 (S); Water Canyon, *Abrams & McGregor* 442, in part (S); Mt. Piños, Ventura Co., *Abrams & McGregor* 233 (S), *Munz* 7034 (P), *Elmer* 4004 (S); San Gabriel Mts., Lytle Creek Canyon, *Hall* 1441 (S); Ice House Canyon, *Parish* 11955 (P,S); Swartout Valley, *Munz* 4644 (P); Bear Valley, San Bernardino Mts., *Munz* 5682 (P); Bluff Lake, *Munz* 8161 (P), *Newsom* in 1926 (P).

Wrightii is most variable in growth habit, making it difficult to key. It seems strange to consider the small 1- or 2-flowered plants collected by *Jones* at Lassen Peak in the same variety with the large Greenhorn Range material of *Hall & Babcock*. This is especially true when one notices the conspicuous bracts on the 1- or 2-flowered specimens; but I am considering the Lassen Peak material and some from Webber Lake collected by *Doten* as atypical and aborted, because in all other small material studied, immature plants of *wrightii* appear this way until the last whorls of flowers are fully developed and then show the lack or minuteness of the upper bracts. Specimens collected from Bluff Lake by *Munz* on June 1, 1924, and by *Newsom* on June 28, 1926, show very well the change of habit which would take place in a month's growth. Specimens from Summit, Placer Co. (*Heller*) also show this variation in the presence of bracts.

C. brachysiphon Eastw. is here reduced to synonymy, the material described being one of the variable smaller forms of *wrightii* not distinct enough for separate varietal rank.

Excluded or doubtful species

C. tenella Benth., in DC. Prodr. 10:593. 1846, equals *Tonella collinsoides* Nutt.

C. floribunda (Gray) Greene, Pitt. 1:55. 1887, equals *Tonella floribunda* Gray.

C. tricolor Rafin. Cat. 13. 1824. Probably *C. verna* Nutt. but am unable to get any information concerning it.

C. barbata Bosse, Verh. Berl. Gartenb. Ver. N. Reih. 1:399. 1853, equals *C. tinctoria* Hartw. according to GRAY Syn. Fl. No. Am. 2:255. 1878.

C. purpurea Rafin., Cincinnati Lit. Gaz. 85. 1824; Atl. Jour. 149. 1832. According to RAFINESQUE's descriptions, *C. purpurea* is much like *C. violacea*, but because of the present range of *violacea*, and due to the fact that no collections of *violacea* have been made east of the Mississippi from the vicinity in which RAFINESQUE states that he found *purpurea*, I feel that what he collected may have been a rather small, uncolored form of *verna*. There is no definite information upon which to base such a decision, however, so *C. purpurea* is placed here rather than in synonymy. *C. purpurea* var. *cuneata* Rafin., *l.c.* and *C. purpurea* var. *parviflora* Rafin., *l.c.* are included in this discussion of *C. purpurea*.

C. hernandezii Elmer, Bot. GAZ. 41:310. 1906. Probably a form of *C. bicolor* var. *typica*, as indicated by description and locality; am unable to locate type material.

POMONA COLLEGE
CLAREMONT, CALIF.

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STUDIES IN CALIFORNIAN HEPATICAE

I. ASTERELLA CALIFORNICA

ARTHUR W. HAUPT

(WITH PLATE X AND TWENTY-ONE FIGURES)

Notwithstanding its large number of species of world-wide distribution, *Asterella* has not received the same critical morphological study which has been given to many of the other Marchantiales. The genus belongs to the Rebouliaceae of EVANS (14), a family including four other well known genera, *Plagiochasma* (*Aytonia*), *Grimaldia*, *Cryptomitrium*, and *Reboulia*. The names *Fimbriaria* and *Hyphenantron* have been in common use for the genus, but both are antedated by *Asterella*. Of the fifteen North American species described by EVANS (9, 14), four occur in California, the commonest one being *A. californica*. The general structural features of this species, especially those of value in classification, are fully presented by HOWE (15) and EVANS (9), while a number of facts relating to its development are given by CAMPBELL (3).

EVANS (14) gives the distribution of *Asterella californica* as "Arizona, California, and Guadalupe Island." According to HOWE (15) it is found in California, "on open or lightly shaded banks, often about rocks, from San Diego as far north, at least, as Mendocino and Shasta Counties." Some of the material used in the present study was collected along a stream bank in Calaveras County, but most of it came from various canyons in the San Gabriel Mountains of Los Angeles County. *A. californica* is the commonest liverwort in the mountains of southern California, being locally abundant on moist banks and canyon sides during the late fall, winter, and early spring. The plants dry up during the long rainless summer, but, as CAMPBELL (3) says, "the ends of the branches remain alive, so that each growing tip becomes the beginning of a new plant." Soon after the plants are revived by the autumn rains, the production of sex organs begins. The most favorable material for the study of their development was collected in October. Although some individuals

may bear mature spores a month or two earlier, the greatest number of plants with ripe sporophytes was found in April.

Another species of *Asterella*, rather common in the San Gabriel Mountains, is *A. palmeri*. In the fall it is difficult, upon superficial examination, to tell this form from the more abundant *A. californica*, but as development proceeds the two may be easily distinguished. The writer has found only these two species of *Asterella* in southern California. In the present investigation *A. palmeri* was used for a study of the development of the embryo, as it was found more favorable for this phase of the investigation than *A. californica*.

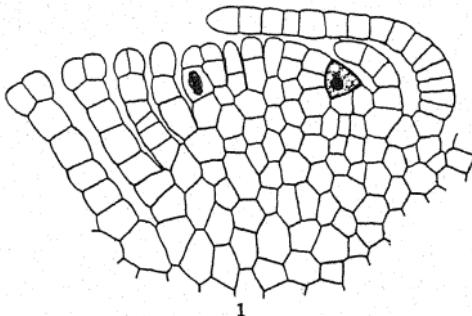


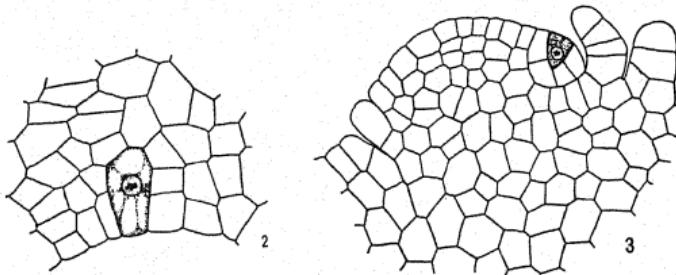
FIG. 1.—Median longitudinal section of sterile thallus, showing apical cell and developing air chambers; $\times 335$.

Except for the account of the embryogeny, however, and in a few other places where specifically noted, all statements in this paper refer to *A. californica*.

THALLUS

The form and structure of the thallus of *Asterella californica* have been accurately described by HOWE (15) and EVANS (9), and most of the facts presented here confirm their observations. The flat, dorsiventral body is green above, but usually slightly purplish below and along the undulate margins. It is rather thick and fleshy, with a conspicuous rounded keel below and on either side, forming a thin wing. There is no dorsal midrib. When dry the margins become strongly incurved. Although some species of *Asterella* produce numerous ventral branches when mature, the branching of *A. cali-*

fornica is apparently always dichotomous. The thallus grows by means of a single large apical cell of the cuneate type characteristic of the Marchantiales (figs. 1, 2). Miss PEISSEL (20), working on *A. blumeana*, reports that the growing point consists of a transverse row of four cells, of which the "apical cell" seen in median longitudinal section is but one member. A careful study was made of this matter, and while occasionally more than one apical cell seemed to be present, because such cases as shown by fig. 2 were so well defined, it was concluded that there is only one apical cell. When growth is vigorous, the immediate lateral segments of the apical cell are difficult to distinguish from the cell itself.



FIGS. 2, 3.—Fig. 2, apical cell of thallus sectioned in plane parallel with dorsal surface; $\times 750$; fig. 3, median longitudinal section of very young carpopecephalum, showing apical cell at forward margin; $\times 335$.

As in all of the Rebouliaceae, the ventral surface bears two rows of appendaged scales, and also both smooth and pegged rhizoids. EVANS (9) says that, in *Asterella*, "rhizoid-initials are apparently not present." With this statement the present study does not agree. Well stained preparations of median longitudinal sections through the apical region clearly reveal the presence of very conspicuous rhizoid initials on the ventral surface, among and just back of the scales which protect the growing point. They are striking because of the unusual density of their protoplasm.

The dorsal region of the thallus consists of loose green tissue, and is sharply distinct from the compact, colorless, ventral region. EVANS (9) correctly describes the situation as follows:

Green tissue rather loose, the air chambers in four or five layers in the median portion, those of the dorsal layer higher and larger than the others but

somewhat subdivided by supplementary vertical partitions often reaching nearly or quite to the epidermis; compact tissue occupying from one-fourth to one-third the thickness of the thallus in the median portion, thinning out gradually on the sides and extending about halfway to the margin.

The air chambers arise schizogenously just back of the apical cell, the split starting at the surface of the thallus and proceeding downward (fig. 1), thus agreeing with CAMPBELL's account (3) and that of Miss PEISSEL (20). Only the first one or two layers of cells are involved, the air chamber being deepened, not by further splitting, but by upgrowth of the surrounding tissue, as the presence of mitotic figures in the epidermal and subepidermal cells clearly shows. This centripetal method of splitting was previously described in *Targionia* by DEUTSCH (4), and later confirmed by Miss O'KEEFFE (19). BARNES and LAND (2) found in an undetermined species of *Asterella* (probably *A. echinella*), as well as in a number of other Marchantiales, that the split is hypodermal in origin and progresses upward. EVANS (8) reports that, in *Grimaldia*, "the place where the split first makes its appearance is not always the same"; it may start either at the surface or below the epidermis. In *Reboulia*, HAUPT (11) describes centripetal splitting, while DUPLER (5) states that the splitting arises both internally and superficially, "generally proceeding from both points of origin simultaneously."

BARNES and LAND (2) also find that, in *Asterella*, secondary splittings occur deep within the dorsal region, the smaller chambers thus formed becoming connected with the primary chambers. Since in the present study no evidence was found of intercellular cleavage, except at the surface of the apical region, the writer is inclined to consider the "secondary" air chambers to be merely extensions of the primary ones. As EVANS (8) reports in *Grimaldia*, the deeper chambers have no supplementary partitions. The cells in this part of the thallus are slightly elongated horizontally, their length being about twice their diameter, and chloroplasts are less abundant here than in the cells above. The single-layered epidermis has thin walls, and is provided with chloroplasts. It is often rich in oil droplets, especially near the growing point. As in the other Rebouliaceae, the thallus air pores are simple, while those on the female receptacle are compound (barrel-shaped).

Intracellular fungi live in the thallus, as in most Marchantiales, and in *Asterella californica* are more or less confined to the upper portion of the ventral region. Here the tissue is colorless and compact, the intercellular spaces being small. Below the fungal zone the cells are elongated horizontally, their length being three or four times their width. They have thin cell walls without pits. EVANS (9) says of *A. californica*, "mycorhiza rarely present in the ventral portion." All of the material used in the present study contained fungi.

RECEPTACLES

While most species of *Asterella* are monoecious, *A. californica* is strictly dioecious. The male receptacle consists of an elongated, median, dorsal disk very slightly raised above the level of the thallus, thus forming a low pad or cushion. If the thallus branches while antheridia are developing, as frequently happens, the receptacle becomes forked; otherwise there is just one growing point behind which antheridia arise in acropetal succession. EVANS (9) notes that the antheridial receptacle of *A. californica* is located at "some distance from the apex." In nearly all of the male plants collected for the present investigation, as well as those observed both under field and cultural conditions, the receptacle begins at the apical notch and in many cases extends the entire length of the thallus. Thus the production of antheridia, after starting, is a continuous process which accompanies apical growth of the thallus. It neither limits vegetative growth nor ordinarily is followed by the formation of a sterile patch, although sometimes there is an intermittent production of antheridia, and then the condition described by EVANS is seen. While LEITGEB (16) regards a male receptacle of the type present in *A. californica* as very primitive, GOEBEL (10) considers it a reduced structure derived from distinctly delimited, well defined receptacles such as *Reboulia* and certain other forms exhibit, and these in turn from stalked receptacles. In *A. palmeri*, which is monoecious, the antheridial receptacle is still less well defined than in *A. californica*.

Each antheridium lies in a pit formed by upgrowth of the tissue surrounding the young antheridium. Mucilage hairs do not arise

within the pit, *Asterella* differing in this respect from *Reboulia*. The top of the antheridial chamber remains open from the beginning. It communicates with the exterior by means of a pore similar to that found on the vegetative portion of the thallus, a feature observed by LEITGEB. The development of the pore margin is also similar to that of the thallus air pores, except that the inner cells do not become so small and pointed.

The female receptacle arises in the apical notch as a small rounded knob, the apical cell being clearly seen at its forward margin (fig. 3). The latter then undergoes two successive dichotomies, thus forming four new growing points. Behind these the archegonia soon appear. As the receptacle becomes conspicuously dome-shaped by intercalary divisions, air chambers appear, and a short stalk is formed with a groove opening anteriorly. Alternating with the archegonia are short rhizoid furrows continuous with the furrow of the receptacle stalk, a feature first noted by LEITGEB. Because in the species which he studied LEITGEB found that the archegonia occur singly, he regarded the female receptacle as a single branch of the thallus, not as a branch system. CAMPBELL has shown, however, that in *A. californica* two or three archegonia are formed in each row in a radial series, and that thus the receptacle is "composite," representing a branch system as in the higher Marchantiaceae. The same condition has been described by ABRAMS (1) for *Cryptomitrium*.

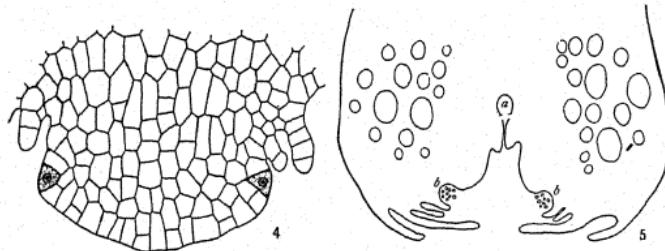
In her recent study, Miss PEISSEL presents an interpretation of the carpocephalum of *Asterella* and related forms not in agreement with that of other investigators, claiming that it arises as a dorsal upgrowth of the thallus, the original apical meristem not undergoing division in connection with its formation. Instead, the meristem remains at the base of the young receptacle, and finally becomes lost in the formation of the stalk.

The mature female receptacle is nearly hemispherical. It is always deeply lobed, the lobes being mostly four in number, frequently five, and rarely six. As a rule only one sporophyte matures under each lobe. The receptacle stalk reaches a length of 10-30 mm., and has but one rhizoid furrow.

FURTHER GROWTH OF THALLUS

Concerning the relation of the formation of a carpocephalum to further growth of the thallus, EVANS (9) writes:

The peduncle arises from the apex of a branch, the growth of which is almost invariably brought to an end. . . . LEITGEB mentions a single specimen of *A. ludwigii* (*Pimeliaria pilosa*) in which an abortive female receptacle failed to limit the growth of a branch, and makes the deduction that such limitation must therefore be a secondary, rather than a primary, result of the development of the receptacle and that the latter is dorsal in origin. No examples of this kind have come to the attention of the writer, but the remarkable conditions sometimes found in *A. californica* may be noted in this connection. In this species,



FIGS. 4, 5.—Fig. 4, section of young carpocephalum cut in plane parallel with dorsal surface of thallus, two apical cells evident; $\times 335$; fig. 5, section of apical portion of young female plant below carpocephalum, cut parallel with surface of thallus: *a*, groove of receptacle stalk; *b*, growing points; $\times 35$.

as figures by HOWE clearly show, the receptacle may grow out from the bottom of a dichotomy, an ordinary branch appearing on each side. This would seem to indicate a dorsal origin, the apical region of the branch continuing its growth but undergoing a dichotomy at once. The subject, however, deserves further study.

While there is reason to believe that this condition is rare in some parts of California, in the southern part of the state it is rather common, and when plants are grown in a moist chamber a number of carpocephala may be produced on the same thallus, especially if fertilization does not take place.

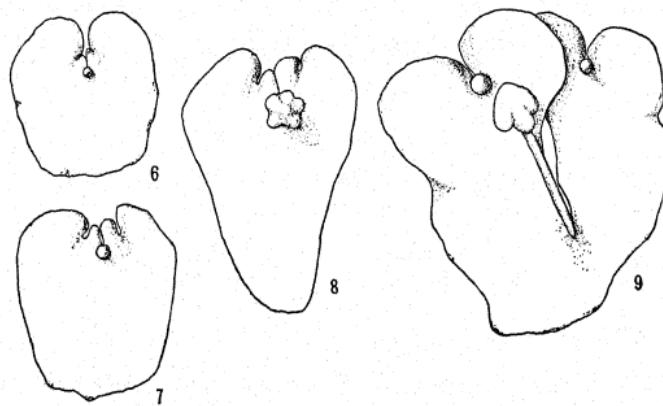
The view that the female receptacle has a dorsal origin is untenable because the apical cell of the thallus is involved in its formation. As already pointed out, CAMPBELL (3) has shown that four (or occasionally five or six) growing points are organized on the

young carpocephalum, each of which gives rise to two or three archegonia in a radial series. The presence of these growing points has been confirmed by the present investigation, and their origin from the primary apical cell of the thallus traced. Fig. 3 shows a single apical cell at the anterior edge of a carpocephalum which is just forming, while fig. 4, cut in a plane parallel with the surface of the thallus, represents a slightly older receptacle in which two apical cells are present. A number of similar stages have been observed. This means that the female receptacle of *Asterella californica* is terminal in origin, and not dorsal as in *Clevea* and *Plagiochasma*. Because the carpocephalum represents a definite branch system, continued growth of the thallus through the activity of the original apical cell is not possible, whereas in cases where it arises as a dorsal upgrowth, the apical cell may continue to function in the usual way.

In forms in which the female receptacle represents a branch system, continued elongation of the thallus may take place by the formation of apical innovations, as in *Preissia* and in certain species of *Asterella*; that is, a single ventral branch may grow out from beneath the receptacle, broadening abruptly and giving the thallus a jointed appearance. In *A. californica*, however, this does not occur. Instead, two new growing points may be organized in the apical notch beneath and just in front of the young carpocephalum (figs. 5-9). As the two lateral branches develop, the receptacle then appears to "grow out from the bottom of a dichotomy," but it should be noted that the receptacle appears before the dichotomy is formed. Each of the branches may again soon give rise to a new receptacle, and the behavior described may be repeated several times. Sometimes one branch becomes suppressed while the other continues to grow, thus giving the thallus an asymmetrical appearance.

It is difficult to determine what factors are responsible for this continued growth of the thallus after a female receptacle has arisen. When plants are grown over the summer in a moist chamber (the period when normally they are dormant in the field) vigorous growth continues, and both male and female receptacles are formed in abundance. The archegonia mature but the antheridia do not, and so fertilization fails to occur. No sperms are discharged from the antheridia of plants grown in a culture chamber, except during the

autumn and winter, which is the time when they mature in the field. When fertilization fails, vegetative growth of the thallus nearly always continues, and the same thing happens when a young female receptacle is excised. Failure of fertilization, then, may stimulate the production of new growing points. It has been observed, however, that sometimes the two new meristems are organized before the archegonia are ripe, and that in the field, as well as under cultural conditions, new receptacles may be formed after sporophytes have developed in the old one.

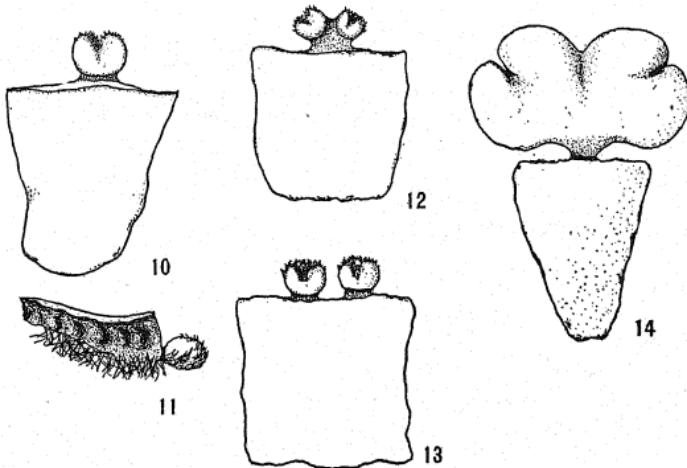


Figs. 6-9.—Series showing dichotomous branching and continued growth of thallus following the appearance of young carpocephalum; $\times 3$.

The present study has furnished evidence in support of the view that the two growing points arising below and in front of the young female receptacle are adventitious in origin, and bear no relation to the original apical cell of the thallus. Young female plants were sectioned longitudinally, transversely, and in a plane parallel to the surface of the thallus, and in the youngest stages careful examination gave no indication of the presence of one or more apical cells except on the receptacle itself. Thus it seems probable that the two growing points which continue the growth of the thallus arise adventitiously at the posterior inner margin of the wings, and just in front of the groove in the receptacle stalk. A large number of stages

similar to that shown in fig. 5 were seen, but in earlier stages no growing points were evident.

Regeneration experiments were performed to determine whether it is possible for new meristems to be organized after the primary apical cell has been removed. Plants were cut transversely a short distance behind the apical region, and the anterior portion was removed. In nearly all cases new growing points made their appear-



FIGS. 10-14.—Regeneration following removal of apical portion of thallus (fig. 11 is side view of fig. 10, fig. 14 is older stage); $\times 3$.

ance within approximately two weeks. The regenerated portion arises from the extreme ventral region of the thallus, and may consist of one growing point or of two (figs. 10-14). Because the formation of adventitious growing points in these experiments is proved, their organization on the uninjured thallus following the utilization of the primary apical cell by the receptacle is rendered probable.

ANTHERIDIUM

So far as the writer has observed, the antheridia develop from segments of the apical cell in strict acropetal succession. The superficial, papillate initial (fig. 22) gives rise to a basal and an outer cell,

the former remaining imbedded in the thallus (fig. 23). As a rule the outer cell gives rise to a filament of four cells by the appearance of three additional transverse walls, the sequence of which was not apparent (figs. 24-26). Vertical walls then appear in two planes at right angles to each other; usually, but not always, they arise first in the lower or middle tiers (fig. 27). Commonly additional transverse divisions follow the appearance of the first vertical walls, and result in the formation of five or six tiers of cells (figs. 28-32). At this point, or slightly earlier, the imbedded basal cell divides, either by a transverse or a vertical wall. This account agrees exactly with that described by the writer for *Reboulia* (11) and *Preissia* (13). In both papers, but especially in the latter, a summary of the literature relating to this phase of the life history is given.

Deviations from the usual sequence of wall formation are uncommon. In rare cases vertical walls may appear when the superficial portion of the antheridium consists of only three cells (fig. 34), or there may be five cells before any vertical divisions have taken place (fig. 35). Although CAMPBELL states that, in an undetermined species of *Asterella* (probably *A. bolanderi*), four or five transverse divisions may be formed before the first vertical walls appear, his figures show only three transverse walls in the superficial portion of the young antheridium (the portion derived from the outer cell). This is the normal situation. DUPLER (6) has demonstrated in *Reboulia*, however, that occasionally four transverse walls are formed in the outer cell at this stage of development.

As a rule the spermatogenous tissue is derived from the three upper tiers of cells in the usual way by the formation of periclinal walls (fig. 33), the lower portion of the antheridium forming the stalk. *Asterella* is similar in this respect to *Reboulia* (11), and to both *Preissia* and *Marchantia* (13). CAMPBELL says that periclinal wall formation in *Asterella* is "exactly as in *Riccia*," where he describes the terminal segments as remaining sterile. Other similar cases are mentioned in the *Preissia* paper (13). CAMPBELL also finds that the top of the mature antheridium is prolonged into a beak or tubular neck. This may happen in some cases, but is certainly not the rule.

ARCHEGONIUM

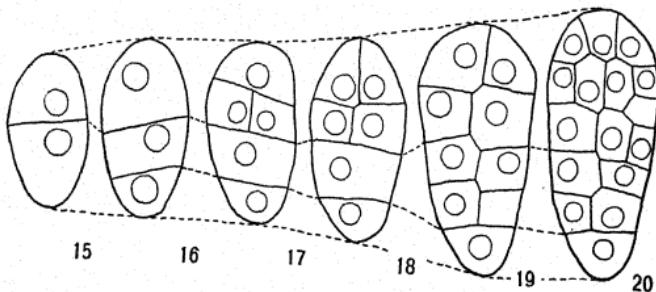
The development of the archegonium is essentially similar to that described by the writer for *Reboulia* and *Preissia*. The earliest stages were not seen. In the youngest stage observed, the primary axial cell and primary wall cells were differentiated, and the basal cell had undergone a transverse division (fig. 36). Further development is typical, the primary axial cell giving rise to the cover cell and central cell (fig. 37), and the latter to the primary neck canal cell and ventral cell (fig. 38). The primary neck canal cell, by two successive divisions, gives rise to four neck canal cells (figs. 39, 40), and then the ventral cell divides to form the ventral canal cell and egg (fig. 41). The cover cell does not divide by a vertical wall until two neck canal cells are formed.

Two cases were seen in which, following the division of the central cell, one of the four neck canal cells had again divided, and in a third instance all of them had divided (fig. 42). CAMPBELL figures an archegonium of *Asterella californica* with an egg, a ventral canal cell, and seven neck canal cells. Thus it seems probable that regularly eight neck canal cells are formed, but that only four are present when the ventral canal cell and egg are differentiated. In *Reboulia* the writer has reported that the number of neck canal cells may later be increased to eighteen or twenty. In *A. palmeri* the central cell divides after four neck canal cells are formed, but no later stages were seen. Variations from the normal development of the archegonium, as found in other Marchantiaceae, have been mentioned in the *Preissia* paper.

EMBRYO

The following account of embryogeny is based on *Asterella palmeri*, as stages illustrating this phase of the life history of *A. californica* were not available. The first division of the fertilized egg is transverse, resulting in two segments approximately equal in size (fig. 15). Two additional transverse walls then appear. In the absence of mitotic figures, their exact sequence could not be established, but it seems likely that a wall appears in each of the two segments. In fig. 16 the hypobasal cell has divided, in fig. 17 the

epibasal cell also. Thus the young embryo consists of a filament of four cells. In this respect *Asterella* resembles *Reboulia* and *Plagiochasma*, but differs from *Preissia*,¹ *Marchantia*, and other forms where an octant stage appears. CAMPBELL says that the latter condition prevails in *A. californica*, but shows just one figure of an embryo too old to prove the case. Miss PEISSEL also describes a similar development for the embryo of *A. blumeana*, but gives no figures at all. In *Reboulia*, DUPLER (7) finds that the sequence of early divisions is similar to that described here for *Asterella*. Wood-



FIGS. 15-20.—Stages in development of embryo of *A. palmeri*; $\times 500$

BURN (21) and HAUPT (12) had previously reported a slightly different sequence, but all three investigators agree that the early embryo consists of a filament of four cells.

Vertical walls intersecting each other at right angles now appear. In *Reboulia* these begin in the lower tiers, but in *Asterella* they seem to start in the upper ones (figs. 17-19). Additional walls are then formed in the upper part of the embryo (figs. 20, 21), the part destined to become the capsule. It is very difficult, perhaps impossible, to relate the early divisions of the embryo to the ultimate differentiation of the sporophyte into foot, seta, and capsule. The writer's study of *Reboulia*, as well as that of WOODBURN, seems to indicate that the foot arises from the hypobasal segment, the seta and capsule

¹ In the writer's paper on *Preissia*, the literature dealing with the embryogeny of the Marchantiales is presented, but mention of MEYER's (17, 18) important studies on *Plagiochasma* and *Preissia* was unintentionally omitted.

from the epibasal. DUPLER (7) is led to believe, however, that both the foot and seta come from the hypobasal cell and just the capsule from the epibasal cell. His interpretation is based on substantial evidence, and may be correct. In *Asterella*, the series of stages presented suggests a similar condition. In certain other Marchantiaceae it has also been shown that the first division of the fertilized egg separates the capsular portion from the rest of the sporophyte.

OLDER SPOROPHYTE

The mature sporophyte and associated structures have been so adequately described by EVANS (9), that little needs to be said in this place. The capsule is nearly spherical, the seta very short, and the foot bulbous. As in all of the Rebouliaceae, the capsule wall consists of a single layer of cells without annular thickenings. In addition to the involucre, a perianth is developed. *Asterella* is the only genus of the Rebouliaceae having this feature. CAMPBELL has studied the development of the spores and elaters, and also spore germination.

Summary

1. *Asterella californica*, the commonest species in California, served as the basis for the present investigation, but was supplemented in part by observations on *A. palmeri*.

2. The fleshy, dichotomous thallus grows by means of a single, large cuneate apical cell. The ventral surface bears two rows of appendaged scales, and both smooth and pegged rhizoids.

3. The green tissue is loose and extensive. The air chambers, lacking chlorophyllose filaments, arise schizogenously, the split starting at the surface of the thallus.

4. The air pores are simple on the thallus and male receptacle, and barrel-shaped on the female receptacle.

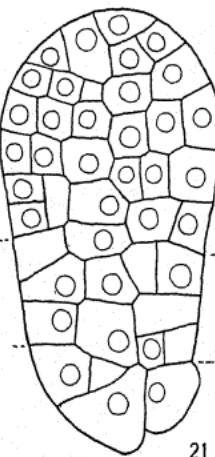


FIG. 21.—Older embryo;
X500.

5. The male receptacle represents a simple dorsal upgrowth of the thallus, the female receptacle a definite branch system, the apical cell of the thallus being involved in its formation. Most commonly four growing points are organized on the latter, and later four conspicuous lobes develop. Ordinarily two or three archegonia arise in each group.

6. Following the formation of a young carpocephalum, a new growing point may appear beneath it at the posterior inner margin of each wing and just in front of the groove in the receptacle stalk. These continue the vegetative growth of the thallus, the receptacle thus appearing to arise from the bottom of a dichotomy.

7. Evidence is presented suggesting that these growing points are adventitious in origin and bear no relation to the original apical cell of the thallus.

8. The development of the antheridium is essentially similar to that of other Marchantiales. Several deviations from the normal sequence of early wall formation are described.

9. Early development of the archegonium presents no unusual features. The ventral canal cell and egg are differentiated after four neck canal cells are formed, but this number is then increased to eight.

10. Before any vertical walls appear, the embryo of *Asterella palmeri* undergoes transverse divisions which result in a filament of four cells. It seems likely that this develops by a transverse division of both hypobasal and epibasal segments of the fertilized egg.

11. It is suggested that the lowest cell of the young embryo gives rise to the foot, the next one to the seta, and the upper two to the capsule.

UNIVERSITY OF CALIFORNIA AT LOS ANGELES
CALIF.

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EXPLANATION OF PLATE X

FIGS. 22-35.—Stages in development of antheridium; $\times 750$.

FIG. 22.—Antheridium initial.

FIG. 23.—First division of same into basal and outer cells.

FIGS. 24-26.—Division of outer cell to form filament of four cells.

FIGS. 27-32.—Formation of vertical and additional transverse walls.

FIG. 33.—Completion of periclinal wall formation.

FIGS. 34, 35.—Unusual stages in early wall formation.

FIGS. 36-42.—Stages in development of archegonium; $\times 500$.

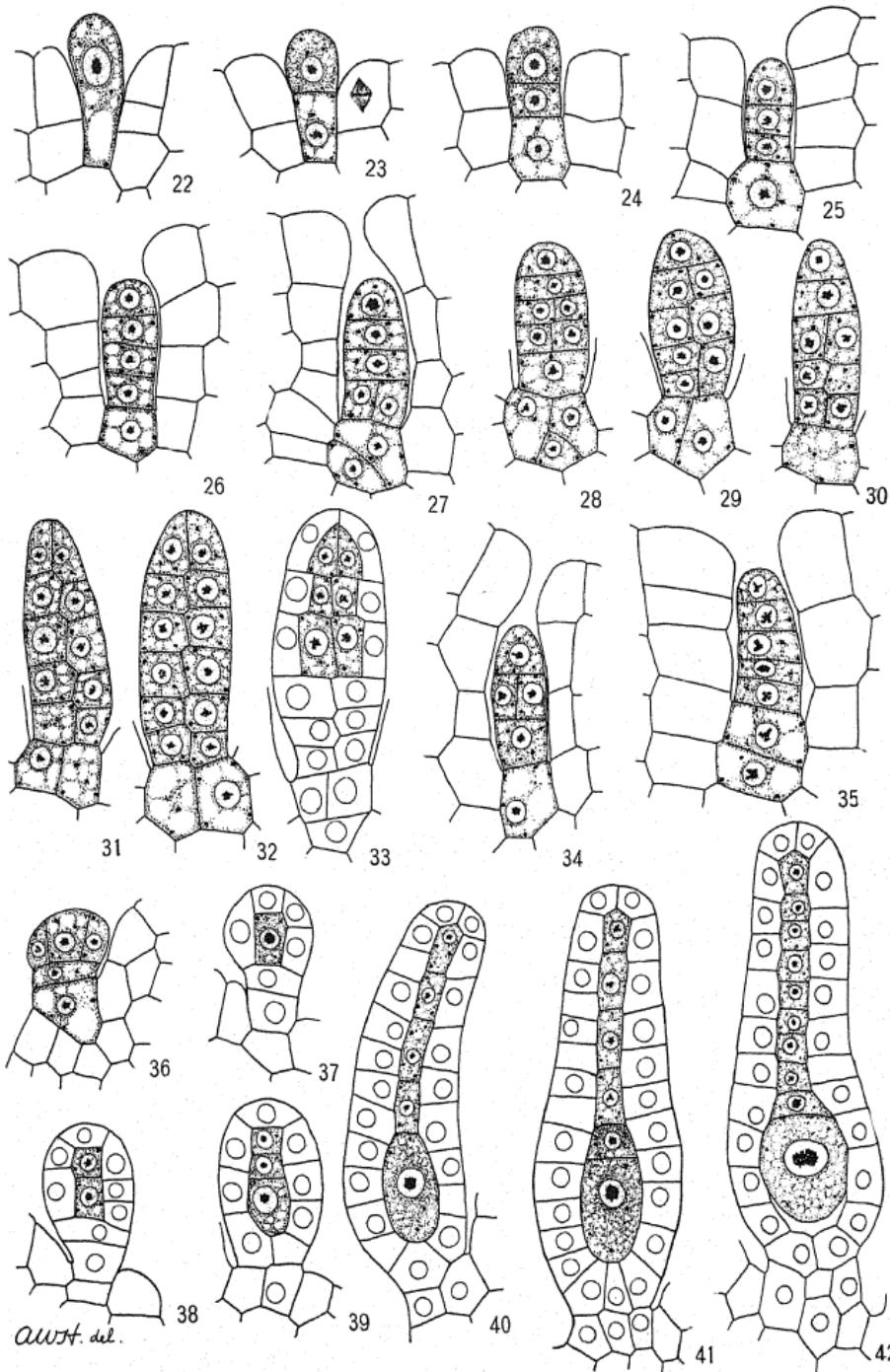
FIG. 36.—Formation of primary wall cells and division of basal cell.

FIG. 37.—Formation of cover cell and central cell from primary axial cell.

FIGS. 38-40.—Division of primary neck canal cell to form four neck canal cells, and formation of vertical wall in cover cell.

FIG. 41.—Differentiation of ventral canal cell and egg from primary ventral cell.

FIG. 42.—Mature archegonium showing increase in number of neck canal cells to eight.





QUANTITATIVE DIFFERENCES IN PALISADE TISSUE IN CITRUS LEAVES¹

F. F. HALMA

It was pointed out in a recent paper by HALMA and HAAS (2) that a greater percentage of the cross-sectional diameter of Eureka lemon leaves is occupied by palisade tissue than is found to be the case with Valencia orange leaves. Since these leaves came from only a few trees growing in the same locality, it was thought of importance to ascertain whether the quantitative difference in palisade tissue between these two citrus species is constant, and to include other species and varieties for comparison.

The data given herewith are believed to be extensive enough to warrant the conclusion that in healthy, fully mature leaves the ratio of the depth of the palisade tissue to the thickness of the leaf is nearly constant for each species.

All measurements were made on free-hand sections cut from the middle portion of the leaf. The values obtained for the depth of palisade tissue are expressed as a percentage of the leaf thickness. The following species and varieties were investigated: Eureka lemon, Lisbon lemon, Rough lemon, citron, Florida sour orange, Marsh seedless grapefruit, Dancy tangerine, Owari Satsuma, two hybrids (Rusk citrange and Sampson tangelo), and *Chalcas exotica* (a citrus relative).

In general arrangement of the leaf tissues these species resemble one another very closely. The majority of the leaves of Eureka and Lisbon lemon trees possess three rows of palisade cells, while as a rule the leaves of the other species and varieties possess only two rows. In many cases the third row is not continuous, hence was not included in the measurements. In nearly all cases there is, abutting against the palisade cells, a continuous row of round cells which were likewise excluded. No relation was found to exist between leaf thickness and palisade development. For example, Eureka lemon and Rough lemon leaves possess practically the same percentage of

¹ Paper no. 189, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

palisade tissue, yet the average thickness of the latter is about 40 μ less than that of the former. Moreover there is a wide range in leaf thickness within the species itself.

The leaf samples of Eureka lemon, Valencia orange, Navel orange, and grapefruit were obtained from young and old trees growing in the coastal and interior regions of southern California, while those of the remaining varieties were obtained only from the interior region.

It is apparent from table I that the values remain nearly constant for each species or variety, regardless of the age of the tree or its geographical distribution. *Chalcas exotica* ranks highest in palisade development, followed closely by the citron and the lemon group. The values then taper off gradually until we come to the Satsuma. The two hybrids are of interest in this connection. The Rusk citrange is a cross between the sweet orange and *Poncirus trifoliata*. The percentage of depth of palisade tissue of the leaves of the latter parent is about 32, but not enough leaves were examined before leaf fall to obtain a statistically reliable figure. Assuming that this number is approximately correct, it would mean that the degree of palisade development of leaves of the Rusk citrange occupies a place half way between that of its two parents. The tangelo, however, which is a cross between the tangerine and the grapefruit, ranks close to the grapefruit in palisade development (table I).

Since the palisade layers are considered to form the chief synthetic tissue, it is logical to assume that the species having leaves with highly developed palisade tissue should have a correspondingly high growth rate. It is a matter of common observation that seedlings of the lemon group grow much more rapidly than those of the orange group. Experiments with leafy cuttings of citrus (HALMA 3) show that rooting depends entirely on the presence of healthy, fully mature leaves. The rate at which the cuttings take root follows very closely the order of the degree of palisade development. Thus it is found that, in the order of rooting ability, *Chalcas exotica* stands at the head of the list, followed closely by citron, the lemon group, and Rusk citrange. The tangerines and oranges are much slower, the grapefruit still slower, and the Satsuma is the most difficult of all to root. Recent experiments with single leaf cuttings have given

TABLE I

DEPTH OF PALISADE TISSUE EXPRESSED AS PERCENTAGE OF LEAF THICKNESS

| VARIETY | SAMPLE | NO. OF LEAVES | MEAN | STANDARD DEVIATION |
|------------------------------|--------|---------------|-----------|--------------------|
| Chalcas exotica..... | 1 | 45 | 31.9±0.21 | 2.1±0.15 |
| Citron..... | 1 | 30 | 30.0±0.31 | 2.6±0.23 |
| | 1 | 29 | 28.6±0.30 | 2.4±0.21 |
| | 2 | 32 | 28.7±0.20 | 1.7±0.14 |
| | 3 | 30 | 29.3±0.19 | 1.6±0.14 |
| | 4 | 30 | 29.4±0.23 | 1.9±0.16 |
| | 5 | 45 | 28.9±0.23 | 2.3±0.16 |
| Eureka lemon..... | 6 | 28 | 28.3±0.20 | 1.6±0.14 |
| | 7 | 25 | 28.8±0.23 | 1.7±0.16 |
| | 8 | 30 | 29.2±0.14 | 1.2±0.10 |
| | 9 | 45 | 28.8±0.18 | 1.8±0.13 |
| | 10 | 30 | 29.2±0.16 | 1.3±0.11 |
| | 11 | 30 | 28.2±0.22 | 1.8±0.15 |
| I-11 | | 354 | 28.8±0.07 | 2.0±0.05 |
| Lisbon lemon..... | 1 | 30 | 29.0±0.28 | 2.3±0.20 |
| Rough lemon..... | 1 | 30 | 28.2±0.23 | 1.9±0.16 |
| | 2 | 30 | 28.9±0.24 | 2.0±0.17 |
| Rusk citrange..... | 1 | 39 | 27.6±0.21 | 2.0±0.15 |
| Dancy tangerine..... | 1 | 30 | 25.0±0.29 | 2.4±0.21 |
| Sour orange..... | 1 | 26 | 24.3±0.23 | 1.8±0.16 |
| | 2 | 30 | 24.8±0.25 | 2.1±0.18 |
| | 1 | 33 | 23.9±0.28 | 2.4±0.20 |
| | 2 | 30 | 23.3±0.16 | 1.3±0.11 |
| | 3 | 37 | 24.7±0.25 | 2.3±0.18 |
| | 4 | 45 | 23.7±0.18 | 1.8±0.12 |
| Valencia orange..... | 5 | 30 | 23.3±0.30 | 2.5±0.21 |
| | 6 | 25 | 24.3±0.36 | 2.7±0.26 |
| | 7 | 30 | 24.3±0.29 | 2.4±0.21 |
| | 8 | 30 | 23.2±0.36 | 3.0±0.26 |
| | 9 | 30 | 23.7±0.34 | 2.8±0.24 |
| I-9 | | 290 | 23.8±0.08 | 3.2±0.06 |
| | 1 | 39 | 23.3±0.25 | 2.3±0.17 |
| | 2 | 30 | 22.8±0.19 | 1.6±0.14 |
| Washington Navel orange..... | 3 | 30 | 22.4±0.25 | 2.1±0.18 |
| | 4 | 30 | 22.6±0.18 | 1.5±0.13 |
| I-4 | | 129 | 22.8±0.12 | 2.0±0.08 |
| | 1 | 30 | 22.0±0.18 | 1.5±0.13 |
| | 2 | 30 | 21.6±0.21 | 1.7±0.15 |
| | 3 | 30 | 22.3±0.26 | 2.2±0.19 |
| Marsh grapefruit..... | 4 | 33 | 21.6±0.19 | 1.6±0.13 |
| | 5 | 33 | 22.0±0.26 | 2.3±0.19 |
| | 6 | 30 | 20.7±0.24 | 2.0±0.17 |
| | 7 | 30 | 21.3±0.26 | 2.2±0.19 |
| I-7 | | 216 | 21.6±0.09 | 2.1±0.06 |
| Sampson tangelo..... | 1 | 30 | 21.6±0.25 | 2.1±0.18 |
| | 1 | 32 | 21.2±0.20 | 1.7±0.14 |
| Owari Satsuma..... | 2 | 30 | 20.9±0.19 | 1.6±0.14 |

similar results. There is the possibility, however, that the leaves of those species which root most rapidly contain more stored carbohydrates than leaves of slow-rooting species. This point needs to be investigated. Unpublished observations show that when rooted cuttings having a similar leaf area are planted in the nursery, the rate of subsequent growth follows the same general order as given for the palisade development (table I). In this connection it is of interest to mention the recent work of ALEXANDROV (1), who found that the ratio of depth of palisade tissue to thickness of leaf differed in the three varieties of grapes which he investigated. He speculates that these findings may be of great practical importance, since the variety

TABLE II

DEPTH OF PALISADE TISSUE; HEAVILY SHADED "ABNORMAL" INTERIOR LEAVES
COMPARED WITH NORMAL LEAVES (30 IN EACH SAMPLE)

| | NORMAL | | ABNORMAL | |
|-----------------------|-----------|--------------------|-----------|--------------------|
| | Mean | Standard deviation | Mean | Standard deviation |
| Eureka lemon..... | 28.6±0.22 | 1.8±0.15 | 21.9±0.22 | 1.8±0.15 |
| Valencia orange..... | 23.7±0.22 | 1.8±0.15 | 19.6±0.25 | 2.1±0.18 |
| Marsh grapefruit..... | 21.6±0.19 | 1.6±0.14 | 19.1±0.28 | 2.3±0.20 |

having the greatest number of palisade cells is the most productive and the one with the least number the least productive.

There are numerous references regarding the influence of environmental factors upon palisade development. For example, LANGNER (4) reports that the palisade tissue is two to three times as thick in a sun leaf as in a shade leaf of *Acer pseudoplatanus*. Citrus leaves collected from the north and south sides of the tree failed to reveal any differences in degree of palisade development; likewise no difference was found between leaves taken from the upper and lower parts of the tree. However, leaves taken from devitalized branches of the dense interior portion showed a marked decrease in the percentage of palisade tissue as compared with the general average. Although mature, these leaves are thin and in many respects appear subnormal. An interesting point observed with this type of leaf is that the lemon leaf still possesses a greater percentage of palisade

tissue than the Valencia, and that the latter exceeds the grapefruit, as is shown in table II.

The effect of soil moisture was not investigated. A possible influence of rootstock on palisade development was considered. Samples of leaves were obtained from Eureka lemon and Valencia orange trees growing on rootstocks of sweet orange, sour orange, grapefruit, and *Poncirus trifoliata*. In addition, leaves were obtained from three different old lemon trees growing on their own roots. The results were the same as those given in table I.

The quantitative difference in palisade development in leaves of different ages was ascertained in the Eureka lemon and is shown in

TABLE III

DEPTH OF PALISADE TISSUE (30 LEAVES IN EACH SAMPLE);
CHANGE WITH AGE OF LEAF (EUREKA LEMON)

| APPROXIMATE AGE (MONTHS) | CONDITION | MEAN | STANDARD DEVIATION |
|-----------------------------|-------------------------|-----------|-----------------------|
| 12..... | Full mature | 29.2±0.16 | 1.3±0.11 |
| 8..... | Full mature | 28.2±0.22 | 1.8±0.15 |
| 5..... | Full-sized, but thin | 26.6±0.16 | 1.3±0.11 |
| 2..... | Full-sized, light green | 23.7±0.30 | 2.5±0.21 |

table III. It will be noted that the two-months-old lemon leaves, which are still immature, average as high as fully mature leaves of the Valencia orange (table I). It is of interest in this connection that cuttings having immature leaves do not root readily. In conclusion it may be mentioned that the quantitative relationship between palisade development and root production of citrus is being investigated.

Summary

1. The ratio of depth of palisade tissue to leaf thickness was found to be nearly constant for each species or variety of citrus examined.
2. Leaves of the lemon group show a value which is approximately 20 per cent higher than that for leaves of the orange. The grapefruit ranks below the orange, and the Satsuma shows the lowest value.

3. The close relationship which exists between the degree of palisade development for each species and its growth rate is discussed.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

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CURRENT LITERATURE

BOOK REVIEWS

Creation by evolution

Probably sufficient popular interest in evolution remains to insure the success of the latest collaborative volume¹ on the subject. This book, under the editorship of FRANCES MASON, is surely authoritative and readable; parts of it are thrilling.

A foreword is provided by HENRY FAIRFIELD OSBORN (Columbia), and an introduction by Sir C. S. SHERRINGTON (Oxford). DAVID STARR JORDAN (Leland Stanford), building upon the isolation which he himself has made famous, concludes that evolution is merely another name for nature. J. ARTHUR THOMSON (Aberdeen) argues that the cumulative effect of the many different lines of evidence forces us to accept evolution; then furnishes the faint-hearted with the following encouragement: "There are great trends discernible in organic evolution, and the greatest of these are toward health and beauty; toward the love of mates, parental care, and family affection; toward clear-headedness and healthy-mindedness; and the momentum of these trends is with us at our best."

H. S. JENNINGS (Johns Hopkins) very simply demonstrates by his own experiments on protozoa that we can actually see evolution occurring among simpler animals.

Following G. H. PARKER's (Harvard) cases of vestigial organs, E. W. MACBRIDE (London) and E. G. CONKLIN (Princeton) divide the task of outlining the embryological evidence for evolution. The former apparently weakens his argument for the sophisticated reader by utilizing inheritance of acquired characters. CONKLIN effectively points his discussion with the following thought: "Religious faith has been able to survive the knowledge of the fact that every human being in the world has come into existence by a process of development. Why should it be supposed that the recognition of an equally natural development of groups of individuals or species would be destructive of religion?"

W. B. SCOTT (Princeton) very temperately undertakes to "support the hypothesis of evolution" with the known facts of present and recent geographical distribution of animals, and more than succeeds. His story of the mammals of the Americas will interest the reader in much the same way as does a historical novel. In the simplest terms, F. A. BATHER (British Museum) explains the validity of fossil evidence.

J. W. GREGORY (Glasgow) gives reasons for abandoning the unnatural and

¹ *Creation by evolution*, edited by FRANCES MASON. 8vo. pp. xx+392. New York: Macmillan Co. 1928. \$5.00.

anti-evolutionary term "species." As a substitute he recommends his own term "circulus," "a natural grouping which has a center of organic stability, and most of its members tend to be near the center so long as the conditions remain the same."

The evolution of animal and plant kingdoms is sketched by Sir ARTHUR SMITH WOODWARD (British Museum) and C. S. GAGER (Brooklyn) respectively. E. W. BERRY (Johns Hopkins) follows with the story as told by fossil plants.

Several special groups of animals are treated in the following chapters. E. B. POULTON (Oxford) has an excellent chapter on butterflies. Surely one must agree that a study of the habits, development, and recent history of this group alone is enough to convince the student of the truth of evolution. Sir A. E. SHIPLEY (Cambridge) follows the evolutionary steps among bees from primitive types to the bumble bee, with its almost human society, and beyond to the honey bee, with its decidedly super-human social organization. W. M. WHEELER (Harvard) tells of the still more remarkable ants, whose evolution had already carried them to a state of social perfection millions of years ago. F. B. LOOMIS (Amherst) gives an animated outline of the fossil records of the horse and elephant. In business-like style, D. M. S. WATSON (London) sketches the evolution of the bird from the lizard ancestor, through the dinosaur-like stage, to *Archaeopteryx*, the toothed fossil bird which was no more efficient at flying than the modern aeroplane, culminating in the bird of the present, which provides a model for the aeroplane of the future.

R. S. LULL (Yale) and W. J. GREGORY (Columbia) divide the task of describing what we know of man's own direct ancestry, and explain why we do not as yet know more. S. K. HOLMES (California) shows us that our simian cousins are more human than we had suspected.

J. S. HUXLEY's (London) evaluation of evolutionary progress is excellent. C. L. MORGAN's (Bristol) vague chapter on "mind in evolution" will prove more useful to the preacher than to the lay reader. In the concluding chapter, H. H. NEWMAN (Chicago) illustrates how the numerous separate lines of evidence for evolution are corroborative, not only in principle but in detail.

Just why no chapter on experimental evolution among higher organisms was included is puzzling to the reviewer. Perhaps the volume was designed to reveal merely what evolution has accomplished, rather than to analyze how the process is carried out.—M. C. COULTER.

Ecological evolution of angiosperms

One of the most stimulating books that has come recently to hand is by BEWES,² well known to all ecologists for his studies of South African vegetation. In the present volume the author attempts to apply the facts and theories of ecology to the problems involved in angiosperm evolution. The whole work is

² BEWES, J. W., *Studies in the ecological evolution of the angiosperms*. 8vo. pp. viii + 134. London: Wheldon & Wesley, Ltd. 1927. 8s.

strongly colored, and naturally so, by BEWS' long African experience. He sees angiosperm evolution largely from the tropical viewpoint, and states that to look at the subject from the viewpoint of the north temperate zone is to glimpse through the wrong end of the telescope. It was in the warmer climates that angiosperms doubtless had their beginnings, and it is in the warmer climates of our present world that we may expect to find the most representative of primitive angiosperms. In Cretaceous times and through much of the Tertiary the climates of the world were largely similar, and moist; but in more recent times have come climatic differentiations in the extratropical regions. It is in these regions that most recent evolution has probably taken place. The author shows that in most of the families and orders of the dicotyledons the tropical forms are more primitive and arboreal, whereas in the temperate zones we have the more recent and herbaceous forms. On the whole the fossil evidence fits in with this idea, although of course there are all too many imperfections in the record. The author discusses with much interesting detail the anatomical and other characteristics of the primitive tropical trees, in contrast with the characteristics of the more advanced types of colder or drier regions. He traces the supposed evolution of lianas from tropical trees, and of epiphytes from lianas. Water plants and annuals are regarded as relatively late products of evolution. Very interesting is his discussion of the Ericaceae. Usually the northern forms in this family are regarded as the main group, and the South African forms are thought to be detached outliers. BEWS thinks, however, that the South African Ericaceae are the more primitive, for they are much more numerous as to species, and in other respects reveal that they are older.

All ecologists should read and digest this interesting volume by BEWS; while it is speculative and at times quite unorthodox, it is suggestive and refreshing.—H. C. COWLES.

Textbook of general botany

A revised edition of the textbook by SMITH, OVERTON, GILBERT, DENNISTON, BRYAN, and ALLEN has just been published.³ The new volume is a revision and enlargement of the first, which was published in 1926. It contains 130 additional pages and nearly 100 new figures. The chapters on roots, stems, leaves, and relations of plants to water have been expanded and many excellent figures added. The treatment of the Thallophytes is one of the noteworthy changes which improve the new edition. In this section the algae are adequately treated; new forms are included in the chapter on green algae; and new chapters have been introduced on the brown and red algae which were discussed very briefly in the first edition. In its present form the book represents a well organized survey of the field in general botany, and should serve as an excellent

³ SMITH, G. M., OVERTON, J. B., GILBERT, E. M., DENNISTON, H. H., BRYAN, G. S., ALLEN, C. E., *A textbook of general botany*. 8vo. x+539. figs. 416. New York: Mac-Millan Co. 1928.

book of reference. It is so arranged that, by selecting to meet individual needs, the book can be used for a semester course in elementary botany.—H. E. HAYWARD.

NOTES FOR STUDENTS

Taxonomic notes.—COKER⁴ has published an account of 15 species of Basidiomycetes, 3 of which are described as new. He also has published a full account of the 8 Chapel Hill species of *Psalliota*.

PENNELL⁵ has described a new *Scrophularia* (*S. oregana*) from Oregon, and a new *Pedicularis* (*P. rainierensis*) from Washington.

PECK⁶ has described new species of *Calochortus*, *Ribes*, *Hydrophyllum*, *Veronica*, and *Hieracium* from Oregon, and also new varieties in other genera.

BROWN⁷ has described a new genus (*Bijlia*) of Mesembryaceae from South Africa. It is based upon a recent specimen which proves to represent a "long lost" species described under another name.

NADSON and KRASSILNIKOV⁸ have described a new genus (*Guilliermondella*) of Endomycetes found in Kalouga, Russia. The description is based on numerous cultures under various conditions.

THÉRIOT⁹ has published the second part of his account of the mosses collected by BROUARD in Mexico, the first part having been published in 1926. He announces that the third part is in preparation. The present part includes 102 species, in 40 genera. The large genera are *Bryum* (14 spp.) and *Brachymenium* (12 spp.). Only 6 species are described as new.—J. M. C.

The middle lamella.—For many years it has been believed by plant physiologists that the middle lamella between plant cells consists of calcium pectate. Three years ago RITTER¹⁰ brought forward evidence that at least in woody tissues it was not even pectic in nature, but consisted of lignin. HARLOW¹¹ has recently confirmed this work, but has shown that in all parenchymatous tissues

⁴ COKER, W. C., Notes on Basidiomycetes and the Chapel Hill species of the genus *Psalliota*. *Jour. Elisha Mitchell Sci. Soc.* 43:233-256. 1928.

⁵ PENNELL, F. W., Notes on Scrophulariaceae of the northwestern United States. *Bull. Torr. Bot. Club* 55:315-318. 1928.

⁶ PECK, M. E., New plants from Oregon. *Torreya* 28:53-57. 1928.

⁷ BROWN, N. E., *Mesembryanthemum* and allied genera. *Jour. Bot.* 66:265-268. 1928.

⁸ NADSON, G., and KRASSILNIKOV, N., Un nouveau genre d' Endomycétacées: *Guilliermondella*. *Comptes Rendus Acad. Sci.*, 187:307-309. 1928.

⁹ THÉRIOT, I., Mexican mosses collected by Brother Arsène Brouard. II. *Smithsonian Miscell. Coll.* 81:1-26. 1928.

¹⁰ RITTER, G. J., The distribution of lignin in wood. *Jour. Ind. Eng. Chem.* 17: 1194-1195. 1925.

¹¹ HARLOW, W. M., The chemical nature of the middle lamella. *N.Y. State Col. For. (Syracuse) Tech. Publ.* 21:1-11. 1927.

it is of pectic substance, as MANGIN first stated. Both these recent workers have relied on solubility tests and not simply on staining reactions, and have shown that our supposedly reliable Ruthenium red is not as reliable a pectic stain as has been supposed.

It may be, however, that we shall have to abandon the view long held that the middle lamella is calcium pectate, although insufficient evidence has been accumulated to prove this point. But the chemists have recently legislated that the middle lamella is protopectin, that is, the pectose of plant physiologists and plant chemists for nearly a century now. In the report of the Committee on Nomenclature of Pectin of the Agriculture-Food Division of the American Chemical Society,¹² the enzyme protopectinase is defined as "the enzyme which hydrolyzes or dissolves protopectin with the resultant separation of plant cells from each other," thus specifically defining the middle lamella as consisting of protopectin. Presumably the evidence considered as justifying this step is the finding that cold alkali oxalates do not dissolve the middle lamella although they dissolve pectates. This is reported, for instance, by CONRAD¹³ and by DAVISON and WILLAMAN¹⁴ recently. On the other hand, plant microchemists have long used dilute acid followed by dilute alkali to dissolve the middle lamella, and this is a treatment of which protopectin should need only the first step.

It will be worth while to abandon the old and established term "pectose" in favor of "protopectin," if thereby we may obtain uniformity of nomenclature; but plant physiologists will regret having the chemical nature of the middle lamella settled for them by legislation rather than by experimentation.—H. S. WOLFE.

North American Flora.—The second part of volume 23 continues the presentation of the Mimosaceae by N. L. BRITTON and J. N. ROSE. The tribe Ingeae is concluded by the presentation of *Lysiloma*, with 31 species, 20 of which are new. The next tribe, Acacieae, includes 12 genera, 8 of which are separated from *Acacia* as new genera, leaving that genus represented by one species. The new genera are *Tauroceras* (2 species), *Bahamia* (1 species), *Feracacia* (2 species), *Lucaya* (1 species), *Fishlockia* (1 species), *Myrmecodendron* (11 species, one being new), *Acaciopsis* (14 species, one being new), and *Acaciella* (49 species, 26 being new). *Senegalia* includes 66 species, 27 being new. The third tribe, Mimosae, includes 12 genera, 4 of which are presented. They are *Leucaena* (39 species, 22 new) and *Acuan* (26 species, 13 new), and the new genera *Ryncholeucaena* and *Caudoleucaena*, each with a single species.—J. M. C.

¹² Jour. Amer. Chem. Soc. 49. Proc. 37-39. 1927. See also the same report quoted in footnote 14.

¹³ CONRAD, C. M., A biochemical study of insoluble pectic substances in vegetables. Amer. Jour. Bot. 13:531-547. 1926.

¹⁴ DAVISON, F. R., and WILLAMAN, J. J., Pectic enzymes. Bot. GAZ. 83:329-362. 1927.

Boron and plant growth.—Recent work has added at least three elements to the original list of ten which research had shown were needed for the growth of plants. These are boron, zinc, and manganese. Of these three elements, perhaps boron has received most attention, at least in recent investigation. The work of such English investigators as WARINGTON, BRENCHLEY, and others, has shown a possible function of boron, by indicating a connection between it and the absorption and utilization of calcium by the plant. Also the work has shown that boron is needed for proper plant development, and to prevent the disintegration of the vascular elements.

JOHNSTON and DORE¹⁵ have recently secured results that are in agreement with these anatomical studies. It was found that the leaves of tomato plants, grown in boron-deficient solutions, had more starch and about twice as much total sugar as plants grown in solutions containing boron; on the other hand, the stems of the boron-deficient plants contained only about two-thirds as much sugar as the normal plants. When boron was lacking in the nutrient solution the plants developed a purple color which was probably due to anthocyanin. It is well known that this pigment often develops to a greater extent in tissue containing a large amount of sugar. Examination by PRIESTLEY of the petioles and stems showed that in the case of the boron-deficient plants the phloem had disintegrated, and seemed to have a decidedly more acid reaction than the phloem of normal plants; so the accumulation of carbohydrates in the leaves would seem to be due to a breakdown of the conductive system. Other characteristics of the boron-deficient plants are noted.—S. V. EATON.

Forest trees of Hokkaido, Japan.—The forests of Japan must be beautiful indeed if their elements are accurately represented in the fine colored plates that comprise the fascicles by MIYABE and KUDO.¹⁶ It is also very convenient to have the descriptions of the various species in English as well as in Japanese. More than a dozen fascicles have already been noted in this journal,¹⁷ and the five now appearing include plates and descriptions of *Magnolia obovata*, *M. kobus*, *Hydrangea paniculata*, *Malus baccata* var. *mandshurica*, *Sorbus commixta*, *Micromelis alnifolia*, *Photinia villosa*, *Crataegus japonica*, *Prunus maximowiczii*, *P. sargentii*, *P. kuriensis*, *P. padus*, *P. grayana*, *P. ssiori*, and *Maackia amurensis* var. *buergeri*.—G. D. FULLER.

¹⁵ JOHNSTON, E. S., and DORE, W. H., The relation of boron to the growth of plants. *Science* 67:324-325. 1928.

¹⁶ MIYABE, K., and KUDO, Y., *Icones of the essential forest trees of Hokkaido*. Publ. by Hokkaido Government, Sapporo 2: Fasc. 15. 27-32. pls. 44-46. 1927; Fasc. 16. 33-38. pls. 50-52. 1927; Fasc. 17. 39-44. pls. 50-52. 1927; Fasc. 18. 45-52. pls. 53-55. 1927; Fasc. 19. 53-58. pls. 56-58. 1928.

¹⁷ BOT. GAZ. 72:55. 1921; 75:431. 1923; 83:328. 1927.

THE
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CYTOLOGICAL STUDIES IN THE BETULACEAE

I. BETULA¹

ROBERT H. WOODWORTH

(WITH PLATES XI, XII)

Introduction

It is now well known and widely recognized that many of the polymorphic groups of plants owe their condition to the readiness with which their species cross. An outstanding and interesting instance is the production, by European monographers (ZAHN 51), of five large volumes devoted entirely to the description of species and variants in *Hieracium*, and the collateral cytological and genetic investigations of ROSENBERG (36, 38) and OSTENFELD (32, 33, 34) which show that *Hieracium* is composed largely of heterozygous forms.

Taxonomic works list several hybrids in both *Corylus* and *Alnus*, and at least fifteen recognized hybrids in *Betula*. Since the group is well known to be polymorphic, it seemed that this very condition might be due to some unrecognized hybridity. This study apparently substantiates such a view.

The birches grow exclusively in the cooler regions of the northern hemisphere, attaining higher latitudes than any other deciduous tree except the mountain ash. From their circumpolar range they radiate out from the north into Europe, Asia, and North America in considerable numbers of parallel forms, which fall into somewhat

¹ Contribution from the Laboratories of Plant Morphology, Harvard University.

definite geographic entities. They are baffling in their variability and very difficult to classify. REGEL, the greatest student of the genus, never used the same treatment twice in his monographic studies. First a group comprised one species, then several species, and finally all phases were considered as species.

Europeans recognize two species, *Betula pubescens* Ehrh. (*B. alba* L.) and *B. verrucosa* Ehrh. (*B. pendula* Roth.). In northern Scandinavia, Iceland, and Greenland are many dwarf forms which are treated as variants of the native species. In Labrador and on Mount Washington and Mount Katahdin are dwarf birches which are called variants of American species. They, however, are not distinguishable from the northern European forms. FERNALD (12) writes of this inconstancy of specific lines:

The dwarf birches like the canoe birches present such tendencies to intergradation that it is difficult to draw clear specific lines between them. . . . In conclusion, it should be emphasized that the specific lines in *Betula*, *Alnus*, *Quercus*, and *Salix* are often too vague. It is quite possible to trace by a series of specimens a direct connection between the dwarf *B. nana* and the tall *B. alba*. Thus *B. nana* in its larger development is separated with difficulty from the Scandinavian *B. alpestris*. This shrub in turn is quite like glabrate states of the American *B. pumila*, which, through its variety *glandulifera*, passes to *B. glandulosa*, the larger developments of which pass in the Cascade Mountains to *B. microphylla*, and in the Saskatchewan region to *B. alba* var. *minor*. The latter shrub is often inseparable on the New England mountains from *B. papyrifera* var. *cordifolia*, which on the lower slopes becomes a large tree and passes gradually to the broad-leaved form figured by MICHaux as *B. papyracea*. A very similar series is readily made to include *B. pendula* and *B. humilis*. But since it is obviously impracticable to regard all these forms as one species, it seems wiser to recognize the more marked centers of variation as species which are admitted to pass by exceptional tendencies to other forms ordinarily distinguished by marked characteristics.

When the members of a large group of plants exhibit such diversity and consequent uncertainty of specific lines that call for frequent revisions, it affords a fertile field for investigations concerning evolutionary tendencies. It has long been known that a number of birch species hybridize more or less freely in nature and under cultivation. JACK (20), ROSENDAHL (39), FERNALD (13), and HELMS and JORGENSEN (19) have contributed important studies on hybrid birches. WINKLER (49) describes eight natural *Betula* hy-

brids and lists six others. They are: *pumila* \times *lenta*, *pubescens* \times *nana*, *verrucosa* \times *humilis*, *pubescens* \times *humilis*, *pubescens* \times *verrucosa*, *nana* \times *verrucosa*, *populifolia* \times *papyrifera*, *papyrifera* \times *pumila* var. *glandulifera*, *lutea* \times *pumila* var. *glandulifera*, *davurica* \times *lenta*, *pumila* \times *nana*, *pumila* \times *glandulosa*, *verrucosa* \times *papyrifera*, and *coerulea*-*grandis* \times *populifolia*. With these conditions obtaining, it seemed that a cytological investigation of the reduction division in species of *Betula* might disclose one of the reasons for their marked variability. This supposition has proved correct, and some interesting results are at hand.

Materials and methods

Material for this study was gathered from labeled specimens in the Arnold Arboretum, where have been brought together a representative collection of the species of *Betula*. Staminate catkins were collected in the early afternoon on warm sunshiny days during the first half of September. The catkins were slit with a sharp razor and immediately put in Carnoy's fluid. By the use of an exhaust pump the air was quickly withdrawn from the tissues to insure rapid fixation. After 24 hours in the fixing fluid the catkins were placed in 95 per cent alcohol, where they remained until the imbedding process.

Experiment proved demineralizing and bleaching to be unnecessary. The material was dehydrated and imbedded in nitrocellulose, which is proving much superior for cytological work than is paraffin. The method employed is that described by JEFFREY (21). Small catkins were treated by the mass method, as described in JEFFREY's (22) report of meiosis in *Drosophila*, wherein numbers of small objects for sectioning are fixed close together on cardboards, stuck with glycerin jelly, imbedded, and sections of a great number of small objects taken with one stroke of the microtome knife.

Sections were cut with a Jung-Thoma sliding microtome about 10 μ in thickness. Thinner sections were not satisfactory because many mother cells were cut, thus making them useless for study. Haidenhain's iron haematoxylin gave the best staining results. COLE's (9) rapid iron haematoxylin technique was also quite satisfactory.

The study and drawing of material were done with a Bausch and

Lomb microscope equipped with a 130 Abbé condenser, a 1.5 mm. 130° Zeiss apochromatic objective, and an 8 power Leitz (periplan) compensating ocular. Strong light was obtained from an 150 watt bulb light. Drawings were outlined from typical stages of pollen mother cells during the reduction divisions with the aid of a camera lucida. All P.M.C.'s. have a thick callose coat, as shown in figs. 33-44. Magnification is the size increase as it appears in print after reduction from the larger original drawings. In the cytological descriptions X indicates the haploid number of chromosomes and $2X$ the diploid.

General microsporogenesis

The meiotic divisions in a normal plant or animal progress quite regularly. In diakinesis the normal homology between paternal and maternal chromosomes brings them together as bivalents. At heterotypic metaphase the chromosomes are arranged definitely, as in fig. 3. At heterotypic anaphase the chromosomes progress in an orderly manner to the poles, as shown in fig. 41. The homeotypic division is not unlike an ordinary mitotic division.

There are occasions when the meiotic phenomena are characterized by abnormalities rather than by the normal division just described. These irregularities in general are due to the disharmony of the paternal and maternal chromosomes when they have their origin in individuals of different species. In other words, irregularities of the meiotic phases are intimately connected with hybridization. An examination of figs. 33-40 makes the scope of these abnormalities quite apparent, and shows the various stages of meiosis in the hybrid *Betula jackii* (*B. lenta* \times *pumila*), which will be discussed in detail later. When the parents of the hybrid differ in their chromosome numbers the meiosis may show marked abnormalities, but if the parents have the same chromosome number, this is not necessarily so; the divisions may be marked by regularity. This brings out the important fact that all hybrids do not exhibit irregular meioses. Also, if they can produce viable seed and proceed to the second filial generation, they may eliminate the causes of abnormalities and become full fledged species which breed true. On the other hand, they may be so sterile that the seed is formed from nucellar budding. These asexually reproducing clones are of course very

stable genetically, and are considered to be good species; but an examination of the reduction division shows that they are heterozygous. The Caninae section of *Rosa* shows this condition very markedly.

Cytology of *Betula* species

DIPLOID SPECIES

SUBSECTION COSTATAE

Betula lenta L., X-14 (meiosis normal).—Fig. 43 shows a heterotypic metaphase plate showing the fourteen chromosomes, which stand unusually far apart, compared with the condition obtaining in other species. Occasionally, however, two of the gemini stand so close together that they appear to be fused into one large mass, as happens so typically in *Corylus*. Fig. 44 shows the initiation of the early anaphase of the first division. Cytomyxis is marked and very common during the prophase, the cytoplasmic strands usually contracting by the time the metaphase is instituted. They are again apparent during interkinesis.

B. nigra L., X-14 (meiosis normal).—The phases of the reduction division compare favorably with those of the preceding species. Fig. 27 shows the heterotypic metaphase plate with fourteen chromosomes. The early homeotypic metaphase seems to be characterized by tardiness of the chromosomes in their action on the spindle. During the prophase there is much cytomyxis between the P.M.C.'s. The reduction division takes place two weeks later than in most species (latter part of September).

B. schmidtii Regel, X-14 (meiosis very abnormal).—When the gemini are forming there is such an incompatibility between some of the chromosomes that they do not form synaptic mates. These then remain as univalents, and cause abnormalities which are known to be intimately connected with plants of heterozygous origin. Fig. 23 illustrates the phase corresponding to the heterotypic metaphase. Ten pairs of chromosomes have united, forming gemini, the remaining eight existing as univalents. Not only the latter but also the bivalents are behaving queerly on the spindle. One of the single chromosomes has left the spindle and is reposing in the cytoplasm; other cells show equally involved conditions. Sometimes there is a

fusion of a few of the chromosomes into a single large mass. Occasionally the heterotypic metaphase appears to be quite normal and the polar view shows fourteen chromosomes (fig. 25). This is an important trait of heterozygotic plants which perhaps is not commonly realized. Along with the abnormalities occur some few normalities, which are capable of producing morphologically good pollen grains. This of course is the reason that the pollen is not entirely sterile in every cross. Instead of the formation of a normal tetrad of nuclei, a polycary mother cell is often formed with five, six, and seven nuclei. Fig. 24 shows such a cell. This polycary may be formed, in part at least, by the migration of whole nuclei from one cell across cytomycotic connections into adjoining cells. The writer has seen unmistakable evidence of this. The pollen appears to be normal. In view of the conditions obtaining in the reduction division of the P. M. C.'s in this species, it is necessary to consider that it may be of hybrid origin.

SUBSECTION ALBAE

$\times B. coerulea$ Blanchard ($B. coerulea-grandis \times populifolia$).—In Gray's manual the following appears under $B. pendula$ Roth: "A polymorphous boreal species, of which the New England phase has recently been designated as $B. coerulea$ Blanchard (blue birch)." Both FERNALD (13) and REHDER (35a) consider this small tree to be a hybrid between $B. coerulea-grandis$ and $B. populifolia$. SARGENT's (41) suggestion, "Perhaps with its variety best considered a natural hybrid between $B. papyrifera$ and $B. populifolia$ " is seen to be quite impossible, since $B. papyrifera$ is a pentaploid species and $B. populifolia$ is diploid. $B. coerulea$ is also diploid. This species is then best considered a natural hybrid between $B. coerulea-grandis$ and $B. populifolia$, both of which have fourteen as the haploid number of chromosomes.

X-14 (meiosis normal).—FIG. 28 shows the heterotypic metaphase plate with fourteen chromosomes. The reduction division is quite regular in its behavior, with the exception that occasionally one or two of the bivalent chromosomes are tardy in their actions on the spindle, and sometimes do not pull apart until the anaphase is well developed. The pollen is normal. The mother cells exhibit

marked cytomyxis and chromatolysis during the prophases. Sometimes the greater part of a nucleus is seen to have crossed the cytoplasmic bridge and is crowding the nucleus of the cell over to the opposite side.

B. coerulea-grandis Blanchard, X-14 (meiosis normal).—Fig. 29 shows the heterotypic metaphase plate with fourteen chromosomes. During the first division the chromosomes are frequently seen to be split for the homeotypic division. Cytomyxis and chromatolysis are marked during the prophases. In one anther a peculiar condition is at hand. Ten mother cells in the prophase have completely fused together, all but the outer cell walls having been dissolved, forming a large continuous mass of protoplasm with chromatin distributed throughout.

B. fontinalis var. *piperi* Sarg., X-14 (meiosis normal).—The chromosome count was made at diakinesis and interkinesis. No phases of the heterotypic division were seen. Both prophases and interkineses were accompanied by marked cytomyxis and chromatolysis. One interkinetic cell had received a whole nucleus from a neighboring cell and the cytoplasmic bridge had practically disappeared. This species has been considered to be a phase of *B. papyrifera*, but that apparently is not the true relation of the plant, because *B. papyrifera* is pentaploid while this appears to be diploid.

B. japonica Sieb., X-14 (meiosis normal).—Fig. 13 shows the heterotypic metaphase plate with fourteen large chromosomes. During the anaphase of this division there appeared many small black bodies in the cytoplasm. These are minute and are not to be confused with chromosomes.

B. japonica var. *mandshurica* (Regel) H. Winkl.—This is apparently a hybrid, since it has forty-two chromosomes as the reduced number rather than the fourteen of the true species. It is a diploid-hexaploid cross, and is treated under the heading "Hybrid tetraploid."

B. pendula Roth., *B. verrucosa* Ehrh.—Although the first name is some three years older, many botanists have called this species by the latter name.

X-14 (meiosis abnormal).—Fig. 30 illustrates a diakinesis with thirteen gemini and two univalents. Many cells in this phase of de-

velopment have been examined. The affinity of the supposedly homologous chromosomes is variable: some cells have eleven bivalents and six univalents; others have twelve bivalents and four univalents; while others are normal with fourteen bivalents. The mother cells with the univalent chromosomes show irregularities during meiosis. The number of lagging chromosomes is directly due to the non-formation of gemini during the prophase. Several anthers contained cells in the homeotypic division which were behaving very queerly. The chromosome masses at the spindle poles were breaking up and migrating across cytomytic bridges into neighboring cells. The quartet of pollen grains is normally formed, but the cells often contain extruded chromosomal bodies. The tapetal cells (sporophytic tissue), while containing twice the number of chromosomes of the meiotic cells, are entirely normal in nuclear division. Fig. 26 shows a group of P.M.C.'s undergoing extreme chromatolysis during the prophase. In three of the cells the nuclei have completely resolved into streaming black strands of chromatin, which sometimes stretch from one cell right across another to a third.

B. pendula has so many phases that it seems quite possible that the specimen on Bussey Hill in the Arnold Arboretum, which was obtained from MEEHAN in Pennsylvania, may be a natural hybrid. This idea is strengthened by the fact that HELMS and JORGENSEN (19) have worked out the cytology of *B. verrucosa*, *B. pubescens*, and many natural hybrids between the two species, all of which are growing in a large bog in northern Sweden. Their results show the meiotic divisions of *B. verrucosa* (*B. pendula*) to be quite regular, with fourteen chromosomes. Thus the cytological evidence at hand indicates that the diversity of the species may be due to its ready crossing with some other diploid species.

B. populifolia Marsh., X-14 (meiosis normal).—Figs. 1 and 2 portray the heterotypic metaphase spindle from side and polar views respectively. Occasionally, during the first division, a bivalent retains its position at the plate until late anaphase, when it separates and its halves go to their respective nuclei. Small dark bodies are seen in the cytoplasm at various stages. They are not chromosomal in nature. Cytomyxis was noted at interkinesis.

SUBSECTION ACUMINATAE REGEL

B. maximowicziana Regel, X-14 (meiosis normal).—Fig. 31 shows the heterotypic metaphase plate with fourteen chromosomes. Cytomyxis occurs at prophase.

TRIPLOID HYBRIDS

SUBSECTION COSTATAE \times NANAE

$\times B. jackii$ Schneid. (*B. lenta* \times *pumila*; diploid \times tetraploid).—JACK (20) describes the origin of this hybrid as follows:

From a vigorous plant of this dwarf birch (*B. pumila*) which was growing about a hundred paces to the east of a number of healthy, well-grown trees of *Betula lenta*, a large quantity of seed was collected in the autumn of 1887. This was sown in January 1888 and resulted in several thousand seedling plants. As these grew, a large number were disposed of or destroyed. Out of several hundred plants which have been kept to maturity, half a dozen individuals show distinct characters, indicating hybridity with *B. lenta*, the only species growing in the vicinity and the pollen of which would be directly blown to the seed plant by the prevailing westerly winds.

JACK also points out the characters of the hybrid, illustrating them along with those of the parents, and showing that they are intermediate in all ways. He continues:

The present instance seems to show how freely the birches may hybridize with each other when two or more species are growing near together and flowering at about the same time. But when growing naturally, although many ovules might be fertilized by pollen from another species, it would be a rare case that one of the perfected seeds should develop into a mature plant as not one seed in tens of thousands gets a chance to grow. On the other hand, under conditions of artificial propagation and cultivation, a large proportion of the seeds sown are expected to develop into plants, and it is under these circumstances that the fact of the frequency of hybridity becomes apparent. It is made especially clear when it occurs among such dissimilar species as *Betula pumila* and *B. lenta*, where the intermediates are so distinct that hasty students might be led to give them new specific names. It may be worth mentioning that Mr. C. E. FAXON and other botanists have found in various parts of New England what appear to be undoubtedly hybrids between *B. papyrifera* and *B. populifolia*.

The cytological investigation of the reduction division in *B. jackii* proves to be a distinct correlation with this report.

X-21 (meiosis very abnormal).—The diakinesis has not been a satisfactory stage for study, on account of the number of chromo-

somes and the small size of the cells. Fig. 33 shows a diakinetic mother cell which appears to have seventeen univalents, twenty-three bivalents, and two trivalents, although each clump must be the product of but one chromosome, because there are just forty-two clumps and there are just forty-two chromosomes from the two parents. This is apparently one of the cells mentioned later in which no reduction takes place. Another cell shows but thirty-five of these clumps, of which seven are bivalents and the remaining twenty-eight univalents. Many of the latter are already split for the second division, however, and appear as if bivalent in nature. This stage of meiosis is typified by abnormality of gemini formation.

Fig. 34 shows the phase just previous to the heterotypic metaphase, with bivalent and univalent chromosomes scattered all along the spindle. Later on the bivalents arrive at the metaphase plate, while the univalents still lag on the spindle; and sometimes, just as the bivalents are breaking, the univalents draw in, so that, if this stage be viewed from one of the poles, a count can be made of the plate. These counts vary extremely according to the number of gemini formed during the prophanes. The most usual condition is that which results from the pairing of the fourteen chromosomes from the pollen parent with fourteen from the seed parent, the other fourteen from the latter remaining univalent. This gives a metaphase plate with twenty-eight (fig. 37) chromosomes, apparently half of them bivalent in nature. Occasionally two or four of the univalents pair, giving rise to one or two extra bivalents, thus making the count of the plate twenty-four or twenty-six. More often all fourteen bivalents are not formed, and the counts are thirty and over. The extreme of this tendency is no pairing of any of the chromosomes. This condition obtains frequently, and of course the cells involved experience no heterotypic division as no reduction takes place. Fig. 35 shows the plate of such a cell, showing clearly forty-two chromosomes. Two-thirds of them are large, apparently having come from the tetraploid parent (*B. pumila*), while the other third are smaller and have come from the diploid parent (*B. lenta*). Since there is no reduction, all of the chromosomes split, and the cell undergoes a mitotic division (homeotypic), producing two diploid nuclei, and subsequently a diad of diploid pollen grains. Fig. 38

shows the metaphase of this single division. The spindle is very broad, and there are no gemini.

Fig. 36 illustrates the anaphase of the first division of one of the heterotypic cells. Univalents are tardy in their movements, and some of the bivalents are undivided. The homeotypic division is likewise typified by lagging of the chromosomes, as shown in fig. 39. Single chromosomes are often extruded into the cytoplasm. Fig. 40 shows polyspory, rather than the normal tetrad, caused by the formation of extra pollen grains from the chromosomes which lagged on the spindles so long that they were not included in the polar nuclei when their nuclear membranes were formed. The pollen grains vary greatly in size, from microcytes to more or less normal-sized grains, and to large double-sized grains. The smaller and some of the larger ones degenerate, causing considerable sterility. ROSENDAHL (39) finds the pollen 25 per cent sterile.

Meiosis and pollen formation are typically normal in both the parents. Fig. 41 illustrates the heterotypic anaphase of the seed parent (*B. pumila*); fig. 42 shows the metaphase plate with twenty-eight large chromosomes. Fig. 43 illustrates the heterotypic metaphase plate of the pollen parent (*B. lenta*), showing fourteen smaller chromosomes. Fig. 44 is the early heterotypic anaphase of the same plant.

The contrast between the normal meioses of the parents and the very irregular meiosis of this hybrid comprises another very distinct proof of the close relation existing between hybridization and resultant meiotic irregularities. The apparent cause of the latter is the lack of homology between the chromosomes during gemini formation. Cytomyxis was frequently noted at prophase.

SUBSECTION ALBAE

B. verrucosa Ehrh. \times *pubescens* Ehrh.—HELM and JORGENSEN (19) are the authors of the taxonomy and cytology of this hybrid. *B. verrucosa* Ehrh. has fourteen gametophytic chromosomes, and meiosis is normal; *B. pubescens* Ehrh. has twenty-eight gametophytic chromosomes, and meiosis is normal. The hybrid between these two species is triploid, with forty-two sporophytic chromosomes. At diakinesis there are fourteen bivalents and fourteen univalents.

During the first and second divisions the univalents are very tardy in their movements on the spindle. The pollen grains show much variation in size and exhibit polycary. One pollen grain is shown with four nuclei. The pollen of the parents is quite normal. HELMS and JORGENSEN present a sketch map of the large bog in northern Sweden where the plants which they studied grew. The positions of the parents and hybrids of the F_1 and F_2 generations are plotted. Drawings of leaves, cone scales, and seeds, and excellent photographs of the individual trees clearly show the intermediate nature of the hybrids. Their paper is altogether a very convincing one.

TETRAPLOID SPECIES

SUBSECTION NANAE

B. pumila L., X-28 (meiosis normal).—Figs. 41 and 42 show the heterotypic anaphase and the metaphase plate respectively. Cytomyxis and chromatolysis occur during the prophase. In one anther several of the P.M.C.'s. had their nuclei sending fingerlike processes into neighboring cells. The chromosomes extended into the extruded portions of the nucleus over into the next cells, but they were never seen to have been extruded into the cytoplasm.

This tetraploid species is an accomplished hybridizer. It is one of the parents in *B. jackii*, *B. sandbergi* (*pumila* var. *glandulifera* \times *papyrifera*), and *B. purpusii* (*pumila* var. *glandulifera* \times *lutea*).

SUBSECTION ALBAE

B. papyrifera var. *cordifolia*. (Regel) Fernald, X-28 (meiosis slightly abnormal).—At diakinesis there is an occasional non-affinity between a few of the chromosomes. This is indicated by from one to six univalent chromosomes lagging on the spindle during the heterotypic division. Fig. 9 shows the metaphase plate of the first division with twenty-eight chromosomes. Many of the mother cells contain very broad spindles. A study of these cells showed them to contain double the number of chromosomes, which appeared to be about half size, suggesting that reduction has failed or that semi-heterotypic division (ROSENBERG 38) has taken place. I have sought in vain for the restitution nucleus of this latter type of division. Counts of the metaphase plates of these cells show them to

contain fifty-six chromosomes (fig. 10). The mitotic division of this cell with the broad spindle results in the formation of a diad of diploid pollen grains. This is discussed under polyploidy.

Cytomyxis and chromosome migration take place frequently. Fig. 11 is a typical example of cells so involved. The chromosome complement is passing across cytoplasmic connections into nearby cells. If the migration is completed and the cells live, we have here one of the possible origins of polyploidy. This was seen in other species and is discussed later.

It is most interesting to note that this variety is tetraploid, while the true species is pentaploid with thirty-five chromosomes as the haploid complement. This is an impossible situation. In any case a difference in chromosome number is certainly adequate evidence for considering the two plants of specific rank. In this case, to be sure, the separation on gross morphological characters is not an easy one, especially in the aberrant forms. This indicates that cytology is coming to be of great importance in clarifying the haziness of the species concept.

B. pubescens Ehrh., X-28 (meiosis normal).—HELMS and JORGENSEN (19) are the authors of the cytology. This species hybridizes readily with the other European species, *B. verrucosa* (*B. pendula*).

HYBRID TETRAPLOID

SUBSECTION ALBÆ × COSTATAE (DIPLOID × HEXAPLOID)

B. japonica var. *mandshurica* Winkl., 2X-56 (meiosis very abnormal).—*B. japonica*, one of the parents of this hybrid, has been noted previously to be very normal as regards meiosis. Fig. 13 shows the heterotypic metaphase plate with fourteen chromosomes.

The meiosis of var. *mandshurica* stands in marked contrast to that of *B. japonica*. The count of the metaphase plate of the first division is forty-two rather than fourteen. There are many univalent chromosomes in evidence during the reduction divisions. Fig. 15 shows a typical metaphase of the first division with these single chromosomes lagging on the spindle. Often these univalents get in to the equatorial plate just previous to the separation of the gemini, so that a chromosome count from the polar view is readily made.

Most of these plates show forty-two chromosomes, fourteen of which are consistently larger than the others (fig. 14). These latter are apparently from the *B. japonica* parent, and are bivalent in nature. The unpaired univalents surround these gemini, numbering twenty-eight. The count varies; sometimes all fourteen of the *japonica* chromosomes do not find mates, thus the count runs as high as forty-five. Then again, not only do the fourteen pair, but so do some of the smaller chromosomes among themselves; thus carrying the count down to thirty-nine. All counts from thirty-nine to forty-five have been made, forty-two being the commonest. Figs. 15 and 16 illustrate two typical phases in the heterotypic division. Large numbers of univalents lag on the spindle. Now a very significant phenomenon takes place. The bivalents and some of the univalents are at the poles of the heterotypic anaphase spindle and incite the formation of nuclear membranes as in the telophase; but so many of the univalents remain on the spindle that these membranes are unable to form through the spindle, and one continuous membrane is laid down around the complete nuclear figure. Between the two poles this membrane is constricted (fig. 47), giving the appearance of the nucleus pulling apart without the formation of a spindle. The nucleus later resumes the usual shape, and of course contains the diploid number of chromosomes. This large nucleus now passes through the second division with but one spindle, and results in the formation of a diad of diploid pollen grains. A large number of these diads appear in the anthers with the tetrads.

When this semiheterotypic division does not take place, the homeotypic division also has lagging chromosomes on the spindle. The mature pollen exhibits a wide range in size. Some of the grains are microcytes, others are larger and more normal, and the diploid grains from the diads are double-sized. A small proportion of the grains are defective. Cytomyxis and chromatolysis are both present to a marked degree during the prophases.

On account of the cytological evidence, this variety must be considered a hybrid between *B. japonica*, which it resembles very much, and some hexaploid species. *B. grossa* appears to be the only known hexaploid species growing in northeastern Asia, but a study of herbarium material produces very little evidence which would

indicate it to be one of the parents. Subsequent study may show *B. davurica*, here considered a dysploid hybrid with about forty-five gametophytic chromosomes, to be involved in the cross.

PENTAPLOID SPECIES

SUBSECTION ALBAE

B. papyrifera Marsh., X-35 (meiosis almost normal).—Figs. 5 and 6 show the heterotypic metaphase from side and polar view, the latter clearly showing the thirty-five chromosomes. Most of the P.M.C.'s divide quite normally. Occasionally there are from one to six lagging univalents during the homeotypic division. Cytomyxis and chromatolysis are marked during the prophase. Many small dark bodies are noted in the cytoplasm. They are not to be considered chromosomal in nature.

This species has been considered part of the true *B. alba* L. In Europe *B. alba* L. is called *B. pubescens* Ehrh. HELMS and JORGENSEN find the latter to be tetraploid, with twenty-eight gametophytic chromosomes; so the supposed affinity is not real. Detailed cytological investigation may serve to help untangle the involved taxonomic conditions of the white birches.

HEXAPLOID SPECIES

SUBSECTION COSTATAE

B. lutea Michx. f., X-42 (meiosis somewhat abnormal).—Diakinesis usually showed forty-two gemini. Occasionally univalents and trivalents were seen among the bivalents. Figs. 7 and 8 show the heterotypic spindle from side and polar views, the latter with forty-two chromosomes; some counts went as high as forty-nine. These were in cells where conjugation did not occur among all the homologous chromosomes. In such cells lagging univalents appeared on the spindles. Several cells were noted which, during the first division, extruded several chromosomes into the cytoplasm. These form microcytes which degenerate. Cytomyxis and chromatolysis are very marked, especially during prophase. Several cases have been noted where the diakinetic nucleus was over against one side of the cell, and the nucleus of the next cell was migrating over a cytoplasmic bridge into the cell in question (fig. 12).

It is likely that this species hybridizes with *B. lenta*. GRAY'S manual states: "Trees with characteristics somewhat intermediate between this and *B. lenta* have been called *B. alleghaniensis*." The cytological findings suggest that *B. lutea* may have a heterozygous complex itself. In fact, many prominent evolutionists now consider polyploidy to be directly due to hybridization.

B. grossa Sieb. et Zucca., X-42 (meiosis normal).—Fig. 32 is the heterotypic metaphase plate with forty-two chromosomes. Peculiarly enough, these two hexaploid species exhibit metaphase plates which are easier to count than some of the diploid species, because the spindles are so much broader and the chromosomes not so close together. Cytomyxis and chromatolysis are marked during the prophase, mother cells often appearing as if the entire nucleus had been lost.

DYSPOLOID SPECIES

SUBSECTION ALBAE \times NANA (PENTAPLOID \times TETRAPLOID)

$\times B. sandbergi$ Britton. (*B. papyrifera* \times *pumila* var. *glandulifera*).—The cytological study of this hybrid has proved to be a distinct correlation and conclusion to the taxonomic studies of ROSENDAHL (39). The latter's observations extended over several years. His drawings of the leaves, catkins, cone scales, and seeds show *B. sandbergi* to possess characters which are intermediate, in every way, between those of the dwarf birch and the paper white birch. ROSENDAHL further points out that there are but three species of *Betula* in Minnesota, where he found this hybrid. These are *B. papyrifera*, *B. lutea*, and *B. pumila* var. *glandulifera*. He has also found and illustrated plants which are undoubtedly hybrids between *B. lutea* and *B. pumila* var. *glandulifera*, and presents photomicrographs of pollen of the three species, each of which shows 100 per cent perfect pollen, and of the two hybrids, which have 33 and 45 per cent of the pollen defective.

The following quotation is from a letter recently received from Professor ROSENDAHL:

I have just completed another paper, which I mailed last week to Dr. ROBINSON, on experimental evidence of the hybrid nature of *B. sandbergi*. Briefly it relates the results of the cultivation of several F_2 plants of the hybrid showing very clear segregation of the characters of both *B. papyrifera* and *B. pumila* var.

glandulifera. Some of the F_2 plants were grown at the Arnold Arboretum from seed which I sent to Dr. SARGENT. Several years later he sent me specimens grown from these seeds and they show very distinct segregation. I have also found that true *B. pumila* does not occur west of New York state and that specimens from Michigan, Wisconsin, Minnesota, and Indiana which simulate *B. pumila* are nothing but F_2 segregates of *B. sandbergi*. I examined last fall the staminate catkins from one of the F_2 plants growing in our garden and found that all of the pollen grains were irregular in shape and probably all entirely defective. Furthermore, all the seeds from the pistillate catkins examined were found to contain no embryos. If you obtained your material from the Arnold Arboretum [which I did] I am wondering if the plants are not from seed I sent to Dr. SARGENT. In that case you have F_2 plants (assuming that my original seed collection was from an F_1 plant).

Cytology of meiosis in B. sandbergi, 2X-63; X-31, 32 (meiosis very abnormal).—A metaphase plate of a tapetal cell showed sixty-three chromosomes. Diakinesis contains so many elements that it is a most difficult stage to study. The *papyrifera* parent of this hybrid has the haploid number of chromosomes (thirty-five). *B. pumila* has twenty-eight. The variety *glandulifera* has not been available yet, but the chromosome counts of *B. sandbergi* indicate that it must be the same as the regular species.

Apparently all of the chromosomes from the *pumila* parent find synaptic mates and appear during the first division as bivalent chromosomes. The remaining seven chromosomes from the *papyrifera* parent either persist as univalents and lag on the spindle; or two, four, or six of them pair among themselves, forming bivalents. When the latter takes place, thirty-one gemini are formed and the heterotypic division is quite normal, there being only one univalent to lag on the spindle. Fig. 48 shows the chromosome complement of one pole of an early anaphase of the first division, with thirty-one chromosomes; fig. 49 shows the other pole with thirty-two chromosomes. There is always at least one univalent, and, unless it splits, the heterotypic division always produces nuclei with different numbers of chromosomes.

More often the seven extra chromosomes, and sometimes many of the others from *B. papyrifera*, do not pair but remain single and lag on the spindle of the first division (figs. 45, 46). Some bivalents are also seen to be tardy. This tardiness of the chromosomes in their movements on the spindle results in the semiheterotypic division

described under *B. japonica* var. *mandshurica*. The nuclear membrane is formed around the whole spindle, making a single large nucleus, as in fig. 47. This nucleus becomes spherical and passes through the second division as one very broad spindle. Figs. 50 and 51 show the metaphase and anaphase of this restitution nucleus. Some chromosomes lag, possibly because they are univalents which split in the first division and will not split again. The product of this semiheterotypic division is a diad of diploid pollen grains (fig. 52). Between 5 and 10 per cent of the mother cells behave in the manner described.

Occasionally the same sort of thing happens in the homeotypic division. Chromosomes lag, as in fig. 53, and the whole complement of each spindle is included in each of two nuclei. This again results in the formation of a diad of diploid pollen grains. Sometimes only one spindle forms this restitution nucleus, the other forming two nuclei as usual. This results in a triad of pollen grains, one diploid and two haploid in chromosome complement (fig. 56).

More abnormalities occur. During homeotypic metaphase the two equatorial plates frequently unite and form one broad spindle as indicated. At anaphase they are still united, and at telophase the nuclear membrane is laid down around the double chromosome masses again, forming two diploid nuclei and subsequently two large diploid pollen grains. Sometimes two of the spindle poles of the homeotypic anaphase fuse (fig. 55), the other two remaining free and forming haploid nuclei (fig. 56). These several methods of forming diads and triads must produce pollen grains with very diverse genotypic complexes. Another peculiarity was often noted during the homeotypic division. A spindle "fiber" stretched between two poles of different spindles and held several chromosomes, as fig. 57 shows.

It is important to note that these irregularities occur only in the cells which undergo the reduction division. The tapetal cells which divide by mitosis are normal in every way (fig. 58). Apparently these abnormalities are due to non-formation of gemini. ROSENDAHL (39) finds the pollen 45 per cent defective. Chromatolysis is frequently seen during prophase.

B. davurica Pall., X-about 45 (meiosis very abnormal).—Material of this plant has been most difficult to fix and stain properly. This is often the case with plants of heterozygous origin. The first division is characterized by many lagging bivalent and univalent chromosomes (figs. 19, 20). This in itself is strong evidence that the plant is of hybrid origin. Fig. 22 is a heterotypic metaphase plate showing forty-five chromosomes. Staining has been so difficult that it has been possible to count but few plates. The one illustrated is very clear and distinct. Others showed from forty-two to forty-nine chromosomes, but they were not always clear. Subsequent investigation will decide the true number.

Broad spindles with double the number of chromosomes appear frequently, and give rise to diads of diploid pollen grains, just as in *B. japonica* var. *mandshurica* and *B. sandbergi*, both of which are hybrids. An attempt to count one of these broad plates showed about ninety chromosomes.

Cytomyxis and chromatolysis are more marked in this species than in any other studied thus far. At prophase it reaches such proportions that whole anthers of P.M.C.'s are seen to be degenerating, all cells being connected by broad cytoplasmic strands. Fig. 21 shows chromosome migration across a cytoplasmic bridge during the first division. The spindle is involved and the cell to the left seems to be losing its entire chromosome complement to the cell on the right. Likewise, during the first division, the pole of a spindle of a cell in anaphase was seen to be connected by a string of chromosomes to the plate of a cell in metaphase. This may be considered a possible method of an increase in chromosome number. Chains of P.M.C.'s occur end to end without any callose sheath separating them.

Soon after the tetrads are formed degeneration sets in. It is very unusual to see a tetrad which appears to be normal. Many microcytes and giant cells occur along with the more normal-sized grains. Fig. 17 shows a giant pollen grain, the product of a whole mother cell; fig. 18 indicates the appearance of most grains. They are highly vacuolate and soon degenerate. These conditions present adequate evidence for the consideration of this plant as a hybrid.

Discussion

Since the beginning of the present century some very definite ideas concerning the formation and evolution of species have been materializing. As early as 1906, MONTGOMERY pointed out that chromosomal relations bid fair to become the basis of taxonomy. By 1910 there were many ideas current concerning the origin of species by hybridization. OSTENFELD (32) reviews these and concludes: "New species certainly arise through hybridization, but this method of the origin of species is limited to certain cases, e.g. *Hieracium*, and is checked in many ways." More recently, the same writer (35) said that species descriptions should be accompanied by cytological investigation and that hybridization probably formed new species in the polymorphic genera.

OSAWA (31), examining species of *Taraxacum*, found much abnormality of meiosis in the parthenogenetic *T. albidum*. He notes that amitotic division of some of the daughter nuclei, lagging chromosomes, dwarf nuclei, and polyspory are common to many hybrids; and further holds that species with large chromosome numbers have been derived phylogenetically from those species containing less numerous chromosomes.

WINGE (48) states:

It must be presumed that occasional hybridization can give rise to the formation of apogamous, or at any rate sexually abnormal "new species," and that these newly formed biotypes and their offspring persist as special minor species or biotypes simply because they are, from their organization, excluded from fertilization, and thus from the adjusting- or mutually supplementing-effect which is, in the writer's opinion, produced by the alternation of generations.

ERNST (11) has written a sizable volume concerning the definite relation between apogamy and hybridity. Apogamous plants are held not to be able to reproduce in the normal manner because they are species hybrids. The two sorts of plants exhibit a cytology which is much alike.

In 1926, DE MOL (30) reported that the first triploid descendants of Dutch flowering bulbs were obtained by union of haploid egg with diploid pollen grain, the latter being produced apparently as a response to physiological conditions induced by artificial shortening of the growing season. He thinks that abnormal physiological states

may play a larger rôle than hitherto supposed in causing chromosome doubling. BLACKBURN and HARRISON (5) explain the formation of a hexaploid rose from crossing a diploid and a tetraploid species. CLAUSEN and GOODSPEED (8), studying *Nicotiana*, found a *glutinosa-tabacum* hybrid (diploid \times tetraploid) which doubled all its chromosomes, showed normal divisions, and produced all perfect pollen. This is a similar case to the well-known *Primula kewensis*, which is a tetraploid hybrid resulting from the doubling of all the chromosomes from the diploid and tetraploid parents (DIGBY 10).

GATES (15) says that the time should come when the description of a species is not complete until the morphology of its chromosome group is known. LONGLEY (27), working with maize, finds a reversal of the general idea, in that the more primitive and less specialized members of maize and its relatives have more chromosomes than the more recent and highly specialized species. The same writer later (29) pointed out that dioeciousness is associated with high chromosome number in *Fragaria*. ROSENBERG (38) stated that there is a cytological basis for a new species if the combining gametes have different numbers of chromosomes and the reduction division splits the whole group in half, as in *Papaver*. SHARP (43) gives an interesting review and discussion of the whole question.

TSCHERMAK and BLEIER (47) studied a hybrid between *Aegilops ovata* (diploid) and *Triticum dicoccoidea* (diploid). In F_5 and F_6 generations there was a doubling of chromosomes and the hybrid is a fertile, constant, and intermediate form which breeds true. GOODSPEED and CLAUSEN (18), experimenting with *sylvestris tabacum* crosses in *Nicotiana*, backcrossed with the *sylvestris* parent and found that three general types were produced: (1) abnormal, almost completely sterile forms; (2) individuals more or less closely approximating the F_1 type; and (3) plants more or less identical with *sylvestris*, showing in some cases a considerable degree of fertility, and self fertilization through a number of generations leads to the establishment of fully fertile races identical with *sylvestris*. The *sylvestris* parent has twelve chromosomes after reduction and the *tabacum* parent twenty-four. Seventeen plants produced by backcrossing with *sylvestris* were examined cytologically. They always showed twelve bivalents and a varying number of univalents, from none to

all twelve, and this series varied all the way from *sylvestris* type to aberrant forms. Such work as this is laying a solid foundation for a more perfect understanding of cytological, genetical, and evolutionary phenomena.

KARPECHENKO (23), reporting results of crossing *Raphanus* with *Brassica*, states:

The tetraploid F_2 hybrids do not show segregation, acquire quite regular reduction division, full fertility, and, moreover, prove unable to cross with one of their parents, *Brassica*. And it seems that we here approach nearer than we ever did the experimental reproduction of one of the processes in species-formation.

So it is quite clear that the recent work is of such a nature that it cannot contain the slightest provocation for questioning its authenticity.

POLYPLOIDY

Some biologists hold the opinion, with GATES (15), that, "In all cases of true polyploidy the increase in chromosome number is by a longitudinal fission of the chromosomes." In many cases, such as in species of *Betula*, it is wholly impossible to ascertain whether or not this condition obtains, on account of the constant size and minuteness of the chromosomes. Also, evidence as to the origin of polyploidy is becoming plentiful, and it does not rely upon the longitudinal fission of chromosomes. I refer to the semiheterotypic division. It is also possible that cytomyxis and chromatolysis may initiate the formation of diploid gametes. Some investigators now believe the underlying principle of polyploidy to rest in hybridization. It is unreasonable then to so restrict, as in the quotation by GATES, the application of a word of such descriptive qualities as polyploidy. Within the last decade or so many outstanding cases of polyploidy have appeared; in fact, it is now seen to be of such widespread occurrence that any genus of a large number of species is justly suspected of containing polyploid members.

GATES (14) studied the behavior of the chromosomes in *Oenothera*. *O. gigas* is a tetraploid form of *O. lamarckiana*, being produced by a duplication of the fourteen chromosomes. This *gigas* form, when crossed with the diploid species *O. lata*, produced a triploid which had twenty-one somatic chromosomes, seven of them from *O. lata* and fourteen from *O. gigas*. The germ cells have ten or eleven

chromosomes, which accounts for the fact that different individuals in a race have different chromosome numbers. At once there is light shed on the origin of polyploidy and dysploidy (numerical changes not involving entire sets).

TAHARA (45) reported from his investigations on *Chrysanthemum* diploid, triploid, tetraploid, and pentaploid species. In the same year TISCHLER (46) produced a remarkable compilation, listing all the chromosome numbers in the plant kingdom which had been determined at the time. No very intensive study is required for one to realize that reduplication of chromosome sets is intimately connected with the cause for the existence of polymorphic groups.

ROSENBERG (36, 37) found the reduced number in *Crepis* to be 3, 4, 5, 8, 9, 12, 20, 21, and in *Hieracium* 9, 18, and 21. Species of *Triticum*, with the fundamental number of seven, occur as haploid, diploid, triploid, tetraploid, and hexaploid (SAKAMURA 40, SAX 42). LJÜNGDAHL (24) found the same sort of double series in *Papaver*. The counts during the heterotypic division showed 7, 14, 21, 35, 11, and 22. LONGLEY (25, 26, 28, 29) has discovered the following haploid numbers, in *Rubus* 7, 14, 21, 28, 35, 42, 56; in *Zea* 10, 20; in *Citrus* 9, 18; in *Fragaria* 14, 21, 28.

TÄCKHOLM's work on the Caninae section of *Rosa* (44) shows diploid, triploid, tetraploid, pentaploid, hexaploid, and octoploid species and hybrids between them in many different ways. AFZELIUS (1) derived some very interesting haploid counts from species of *Senecio*: 5, 10, 20, 25, 30, 90, also 18, 19, 24, 25, 29. The forms with the latter counts are undoubtedly derived from gametes formed by irregular divisions. CLAUSEN (7), in his recent study of *Viola*, reports slight deviations from the cardinal numbers to the following extent: 6, 7, 10, 11, 12, 13, 17, 18, 20, 24, 26, 27, 30, 36.

ORIGIN OF POLYPLOIDY

Non-reduction.—BELLING and BLAKESLEE (3, 4) report the absence of the heterotypic division in all four types of *Datura*: haploid, diploid, triploid, and tetraploid. They show that the occurrence of the phenomenon can be intensified by cold. The homeotypic division results in the production of two diploid nuclei which form a diad of large pollen grains.

CHIPMAN and GOODSPEED (6) discuss the cytological features of a *Nicotiana tabacum* var. *purpurea* haploid with twenty-four univalent chromosomes. Often none of them forms bivalents, and the splitting of all the univalents at the first division produces diads of pollen containing the full haploid set of chromosomes.

In \times *Betula jackii*, as noted, there are some P.M.C.'s in which the non-affinity of the chromosomes is accentuated to the extent that no pairing takes place; consequently a broader spindle is formed. All the chromosomes split in this first division, forming a diad of diploid pollen grains.

In *Betula papyrifera* var. *cordifolia*, P.M.C.'s frequently are seen to hold broad metaphase and anaphase plates which contain double the number of chromosomes. Diads of diploid pollen grains are formed. Whether this is to be considered a case of non-reduction is not yet certain. There is evidence that one mother cell may receive the chromosome complement of another by cytomixis. The diads may also be the consequence of semiheterotypic division, although I have been unable to find any restitution nuclei.

Betula davurica, as noted under the cytology of species of *Betula*, shows exactly the same conditions as *B. papyrifera* var. *cordifolia*. It also produces giant pollen grains, which are tetraploid rather than haploid, being the product of whole P.M.C.'s.

Semiheterotypic division.—ROSENBERG (38) describes this phenomenon. The heterotypic division is prematurely interrupted:

Round the entire spindle figure a new nuclear wall is formed, resulting in the production of a single large nucleus, the chromosomes of which divide quite in the same manner as in normal interkinesis, but with the diploid number of chromosomes. Such P.M.C.'s divide only once and become dyads thus producing pollen cells with the diploid number of chromosomes. . . . The cells of the dyads, with the diploid number of chromosomes, very often develop into mature pollen with normal exine.

ROSENBERG worked this out in *Hieracium*.

KARPECHENKO (23) has executed some interesting experiments in crossing *Raphanus* and *Brassica*, and concludes with reason that he has "experimentally reproduced one of the processes in species formation." The F_1 hybrids exhibit P.M.C.'s which, in some cases, have no union of the nine chromosomes from each parent. The

univalents lag on the spindle, and, except on occasional extrusion, are all included in one nucleus rather than two nuclei. All the chromosomes now divide on one large spindle, forming a diad each cell of which has the somatic number of chromosomes, eighteen. Occasionally irregularities of distribution cause the two pollen grains of the diad to contain seventeen and nineteen, or sixteen and twenty chromosomes.

KARPECHENKO further finds that in the archesporial cells a nuclear division sometimes takes place without cell division. The chromosomes in neither nucleus conjugate, and at the first division the two spindles fuse into one large one. The chromosomes lag so that all are included in one nucleus. At the second division the univalents all split, giving thirty-six chromosomes in each cell of a diad. These pollen grains are tetraploid. A few univalents split at both divisions, making a few extra, and a few are at times extruded so that the number is thirty-six, more or less. KARPECHENKO also finds triploid gametes to be produced. He crossed the *Raphanus-Brassica* hybrid back with *Raphanus*, producing a triploid. The P.M.C.'s showed abnormalities such as lagging and chromosome extrusion. Often the heterotypic division was of the type under discussion and resulted in the formation of a restitution nucleus. The homeotypic division of this nucleus produced a diad of triploid gametes. Although KARPECHENKO finds the triploid and tetraploid forms to occur most frequently in his cultures, there also appear plants with the following constitution: 27-28, 29, 36-38, 38, 40-42, 45, 51-53, 51.

This study is one which provides proof for one of the theories of the origin of polyploidy, namely, hybridization. Its value is accentuated in that it also shows the method involved. The semiheterotypic division is found to produce diploid pollen in two species, the hybrid *B. sandbergi* and the apparent hybrid *B. japonica* var. *mandshurica*. In *B. sandbergi* a restitution nucleus is formed, not only about the first spindle, but also about the homeotypic spindles in cells which complete the heterotypic division, which again results in the production of a diad. In this *B. lenta-pumila* cross the diads make up some 5-10 per cent of the pollen. In *B. japonica* var. *mandshurica* diads often appear in number equal to tetrads.

Other investigators have noted the formation of diploid pollen grains by this semiheterotypic division. BEER (2) worked with *Fuchsia*; YASUI (50) artificially raised *Papaver* hybrids; LJÜNGDAHL (24) studied *Papaver*; CHIPMAN and GOODSPEED (6) investigated the *Purpurea* haploid of *Nicotiana tabacum*.

Diploid gametes by fusion of homeotypic spindles.—This is described under *B. sandbergi*. It has also been noted by ARZELIUS (1) in *Senecio*, KARPECHENKO (23) in *Raphanus* \times *Brassica*, and by GOODSPEED and CLAUSEN (18) in *Nicotiana*.

Cytomyxis and chromatolysis.—Cytomyxis is protoplasmic continuity, usually in the form of strands, between P.M.C.'s. Apparently it is a widespread condition, which of itself is of no special import. It may be enlarged or aggregated plasmodesmae. Chromatolysis is the dissolution of the karyoplasm. When these two conditions obtain at the same time, and elements from nuclear breakdown migrate across protoplasmic bridges into adjoining cells, the correlation becomes of importance. During the prophases the nucleus is frequently seen to send out long chromatic prolongations which extend across the protoplasmic connections into neighboring mother cells; and occasionally right across the second cell into a third, where it usually ends in a terminal enlargement (fig. 26). I have noted such states in practically all of the species of *Betula* investigated. This has often been reported, the general opinion being to consider such nuclear migrations as abnormalities incited by the action of the fixing fluids.

When a part or the whole of a chromosome set migrates across cytomyctic strands into adjacent cells during the active phases of meiosis, as shown in figs. 11, 12, and 21, it is tempting to look upon such actions as possible cause for the production of other than haploid gametes. In *B. schmidtii* polycary is caused not only by nuclear extrusions, but also by the migration of whole nuclei from cells in the homeotypic telophase across protoplasmic bridges into neighboring cells. Much more observation on these phenomena is necessary before their full significance can be realized.

Giant pollen grains.—The giant grains considered under *B. davurica*, if they affected fertilization, might produce various cases of polyploidy.

DYSPOLOIDY AND ITS ORIGIN

Dysploidy has often quite apparently arisen from the polyploid species; from one of the gametes having one or two chromosomes (more or less) than the haploid number, due to unequal distribution. In the case of *B. sandbergi*, the combination of tetraploid and pentaploid species could result only in a dysploid species.

KARYOPLASMIC RATIO

Many investigators have noted that a definite relation appears to exist between the number of chromosomes, the size of the nucleus, and the size of the mother cell. Figs. 1-8 clearly illustrate this condition in four of our common northeast American species of *Betula*.

Summary

1. Polymorphic groups of plants are consistently proving to contain species which hybridize readily.
2. A wealth of evidence indicates hybridism to be the cause and polymorphism to be the effect.
3. *Betula* is a highly polymorphic genus.
4. Polymorphic groups usually show polyploidy.
5. *Betula* is a polyploid genus containing diploid, triploid, tetraploid, pentaploid, hexaploid, and dysploid species and hybrids.
6. Species of *Betula* are known to hybridize very readily.
7. The correlation of taxonomic and cytological investigations of $\times B. jackii$ and $\times B. sandbergi$ presents complete documents of natural hybridization. All of the abnormalities of meiosis which have been considered as characteristic of hybrids have been found to occur in these two species crosses.
8. Hybridization leads to the production of polyploid gametes by the very interesting semiheterotypic division and by non-reduction; consequently heterozygosis is to be considered one of the methods of the origin of polyploidy.
9. Plants, commonly recognized as typical of the following species, are found to exhibit the abnormalities of meiosis which are characteristic of known hybrids and are therefore held to have arisen by heterozygosis: *B. japonica* var. *mandshurica*, *B. davurica*, *B. pendula*, and *B. schmidtii*.

10. Polymorphism in *Betula* is apparently due to the readiness with which the species cross in nature.

11. It follows that *Betula* is another genus in which the multiplication of species has come about, partly at least, by hybridization.

LABORATORIES OF PLANT MORPHOLOGY
HARVARD UNIVERSITY

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EXPLANATIONS OF PLATES XI, XII

Phases of microsporogenesis in *Betula* species; $\times 2200$ unless otherwise stated. Note proportional increase in cell size and breadth of equatorial plate as the number of chromosomes increases from 14 to 28 to 35 to 42 in four of our common northeastern species of *Betula* (figs. 1-8).

PLATE XI

Betula populifolia Marsh.

FIG. 1.—P.M.C. at heterotypic metaphase.

FIG. 2.—Same from polar view showing 14 chromosomes.

Betula pumila L.

FIG. 3.—P.M.C. at heterotypic metaphase.

FIG. 4.—Same from polar view showing 28 chromosomes.

Betula papyrifera Marsh.

FIG. 5.—P.M.C. at heterotypic metaphase.

FIG. 6.—Same from polar view showing 35 chromosomes.

Betula lutea Michx. f.

FIG. 7.—P.M.C. at heterotypic metaphase.

FIG. 8. Same from polar view showing 42 chromosomes.

Betula papyrifera var. *cordifolia* (Regel) Fernald

FIG. 9.—Heterotypic metaphase plate showing 28 chromosomes.

FIG. 10.—Metaphase plate of cell which did not complete reduction division, showing 56 chromosomes.

FIG. 11.—Three cells undergoing chromatolysis during homeotypic division, showing migration of chromosomes; $\times 1600$.*Betula lutea* Michx. f.FIG. 12.—Cytomyxis and chromosome migration, one cell losing its chromosome complement to its neighbor; $\times 1600$.*Betula japonica* Sieb.

FIG. 13.—Heterotypic metaphase plate showing 14 large chromosomes.

Betula japonica var. *mandshurica* (Regel) H. Winkl.

FIG. 14.—Heterotypic metaphase plate showing 42 chromosomes, 14 of them large.

FIG. 15.—Heterotypic metaphase showing lagging univalents; $\times 1600$.FIG. 16. Heterotypic anaphase showing lagging chromosomes; $\times 1600$.*Betula davurica* Pall.FIG. 17.—Giant pollen grain, product of whole mother cell; $\times 500$.FIG. 18.—Pollen grain normal in size, highly vacuolate; $\times 500$.FIG. 19.—Heterotypic metaphase showing lagging bivalents and univalents; $\times 1600$.FIG. 20.—Heterotypic anaphase showing lagging bivalents and univalents; $\times 1600$.FIG. 21.—Chromosome migration between two cells during heterotypic metaphase; $\times 1600$.

FIG. 22.—Heterotypic metaphase plate showing 45 chromosomes.

Betula schmidii Regel

FIG. 23.—Phase corresponding to heterotypic metaphase showing bivalents and univalents lagging all over spindle.

FIG. 24.—Polycaric mother cell resulting from chromosome extrusion.

FIG. 25.—Heterotypic metaphase plate of normal division showing 14 chromosomes.

Betula pendula Roth.FIG. 26.—Chromatolysis in mother cells previous to heterotypic division; $\times 1100$.*Betula nigra* L.

FIG. 27.—Heterotypic metaphase plate showing 14 chromosomes.

Betula coerulea Blanchard

FIG. 28.—Heterotypic metaphase plate showing 14 chromosomes.

Betula coerulca-grandis Blanchard

FIG. 29.—Heterotypic metaphase plate showing 14 chromosomes.

Betula pendula Roth.

FIG. 30.—Diakinesis showing 14 pairs of chromosomes.

Betula maximowicziana Regel

FIG. 31.—Heterotypic metaphase showing 14 chromosomes.

Betula grossa Sieb. et Zucc.

FIG. 32.—Heterotypic metaphase plate showing 42 chromosomes.

PLATE XII

Betula jackii Schneid. (*B. lenta* \times *pumila*)

FIG. 33.—Diakinesis showing univalent, bivalent, trivalent, and quadrivalent chromosomes.

FIG. 34.—Phase corresponding to heterotypic metaphase, chromosomes scattered all along spindle (note bivalents and univalents).

FIG. 35.—Heterotypic metaphase plate showing 42 chromosomes, about two-thirds of them large, the others smaller.

FIG. 36.—Heterotypic anaphase showing lagging univalents and undivided bivalents (non-disjunction).

FIG. 37.—Heterotypic metaphase plate showing 28 chromosomes.

FIG. 38.—Metaphase of second division of cell with broad plate containing unreduced number of chromosomes.

FIG. 39.—Homeotypic anaphase showing lagging chromosomes.

FIG. 40.—Polyspory: 6 vari-sized pollen grains arising from one mother cell.

Betula pumila L., maternal parent

FIG. 41.—Heterotypic anaphase (note regularity).

FIG. 42.—Heterotypic metaphase plate containing 28 large chromosomes.

Betula lenta L., paternal parent

FIG. 43.—Heterotypic metaphase plate showing 14 small chromosomes.

FIG. 44.—Heterotypic metaphase (note regularity).

Betula sandbergi Britton (*B. papyrifera* \times *pumila* var. *glandulifera*); $\times 1600$

FIG. 45.—Heterotypic metaphase showing lagging bivalent and univalent chromosomes.

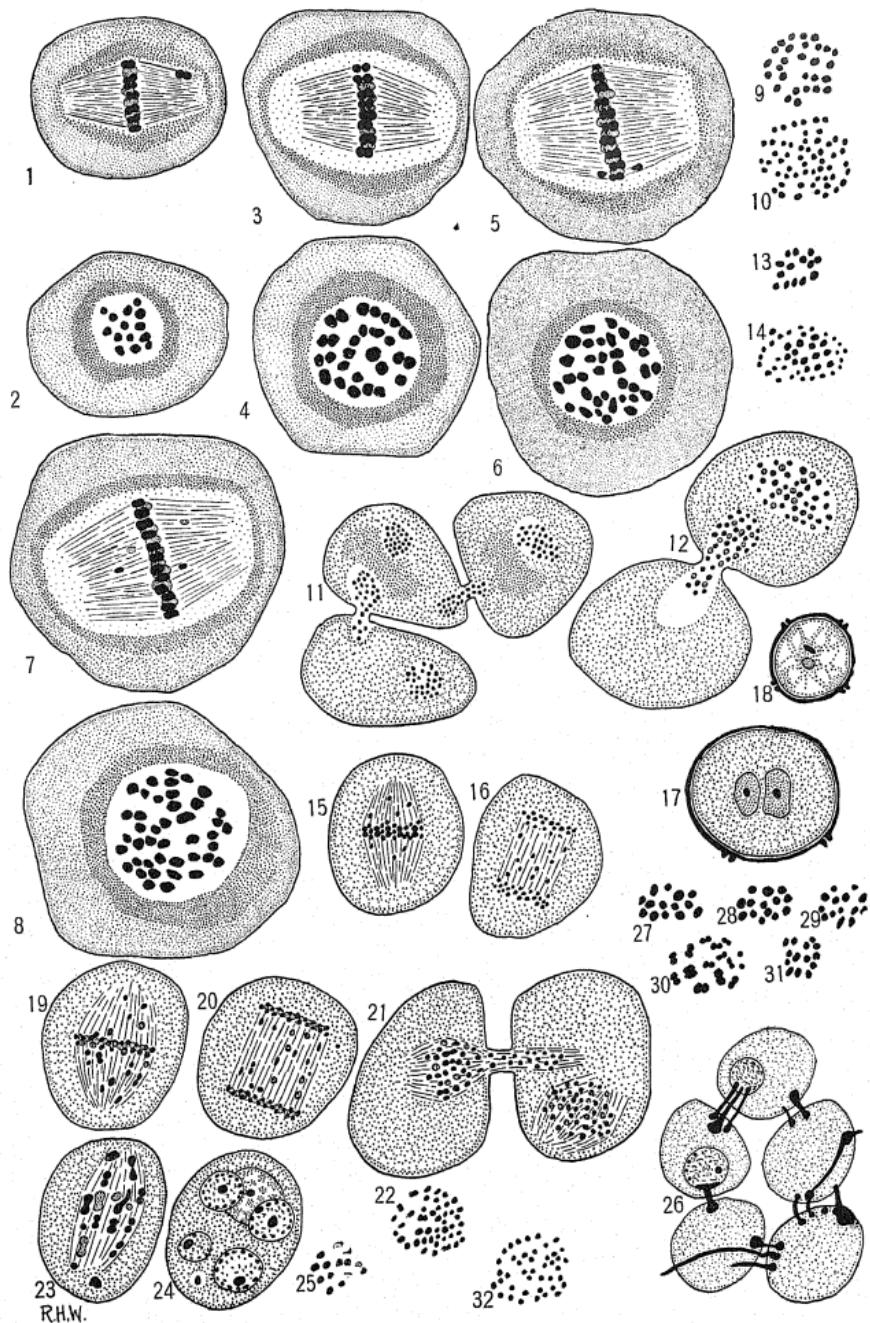
FIG. 46.—Heterotypic anaphase showing lagging bivalent and univalent chromosomes.

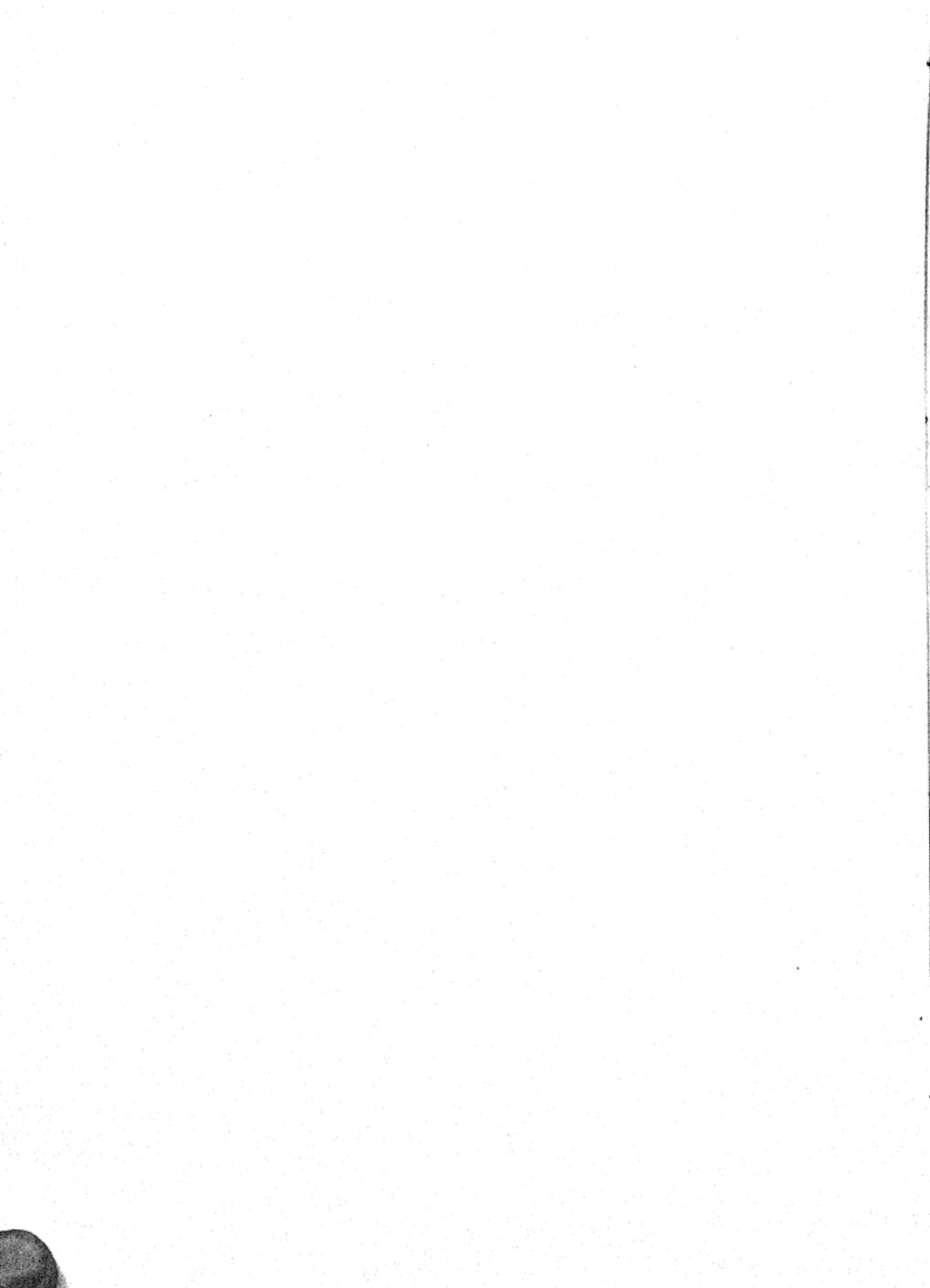
FIG. 47.—Restitution nucleus near close of semiheterotypic division.

FIG. 48.—Heterotypic early anaphase plate with 31 chromosomes.

FIG. 49.—Same with 32 chromosomes.

FIG. 50.—Metaphase of second division in cell containing unreduced nuclear complement, chromosomes which would normally occupy two spindles are all on one very large spindle.





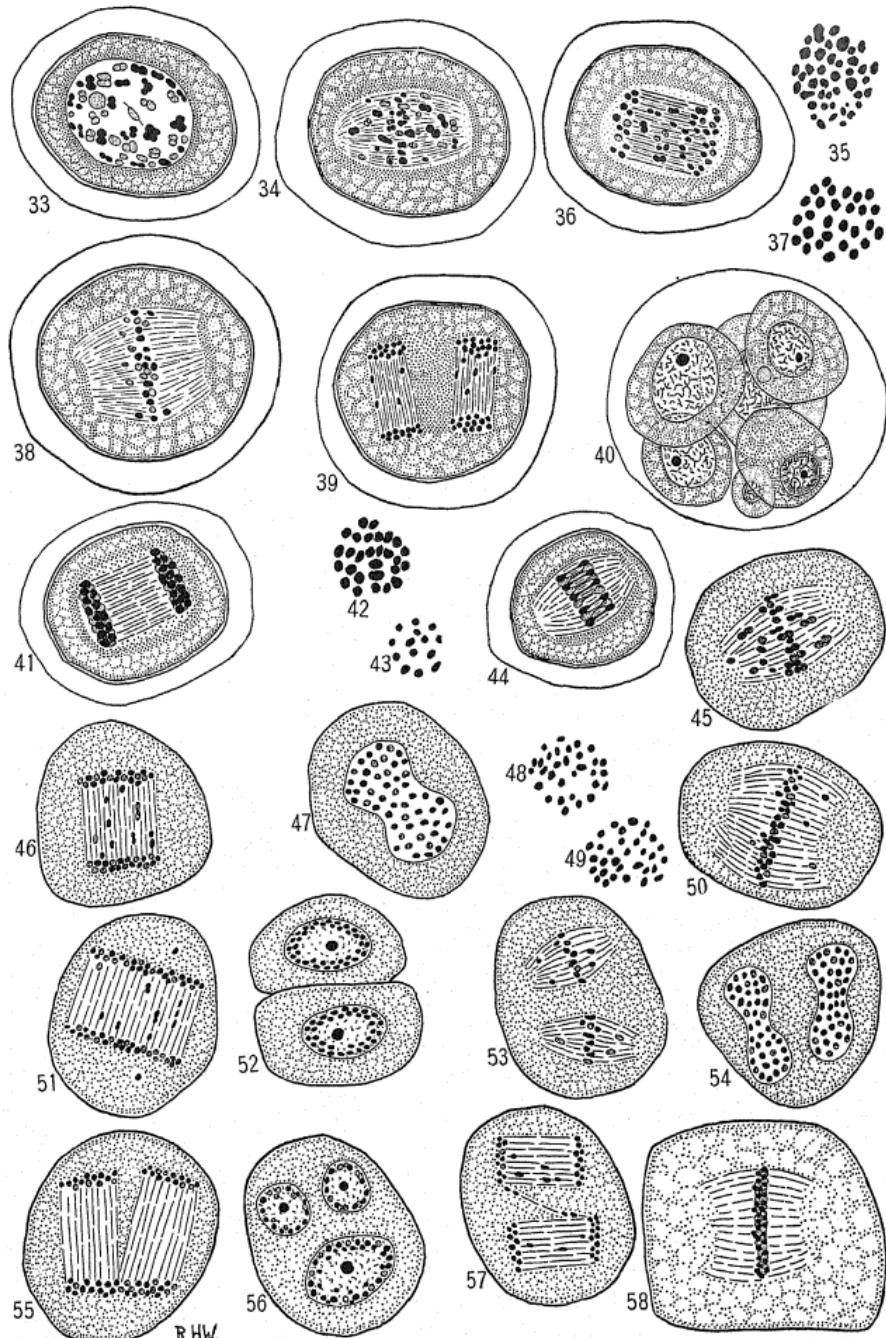




FIG. 51.—Anaphase of broad plate containing unreduced nuclear complement (note many lagging chromosomes).

FIG. 52.—Diad of diploid pollen grains; product of semiheterotypic division.

FIG. 53.—Homeotypic metaphase of cell not undergoing semiheterotypic division; chromosomes lagging.

FIG. 54.—Two restitution nuclei of a semihomotypic division.

Fig. 55.—Homeotypic anaphase showing nuclear complement of two poles fused.

FIG. 56.—Result of fusion showing one diploid nucleus and two haploid nuclei.

FIG. 57.—Homeotypic anaphase showing chromosomes lagging on spindle and peculiar connection of the two spindles by a "fiber" which holds four chromosomes.

FIG. 58.—Metaphase of tapetal (sporophytic) cell (note characteristic normality of division).

COMPOSITION OF WALNUT TREES AS AFFECTED BY CERTAIN SALTS¹

A. R. C. HAAS

(WITH THREE FIGURES)

Introduction

It is a matter of common observation that walnut trees are extremely sensitive to accumulations of certain salts in the soil. The accumulation in soils of unfavorable salts, such as chlorides or sulphates, may result from frequent irrigation with saline water, faulty fertilizer practice, as well as poor drainage, etc. Under such conditions the cycles of growth may be so short as to give the tree a stunted appearance. In extreme cases the trunks fail to increase appreciably in diameter, and together with the branches they may be covered with a dense growth of lichens.

The leaves of walnut trees growing in soil containing considerable saline material may be undersized and may have a wilted appearance. The leaves are most frequently burned along the tip and margins and in extreme cases are also burned between the veins. Premature abscission of the leaves may occur, and this often leads to the production of late growth which matures poorly and is very susceptible to injury by freezing.

The tree materials studied in the present investigation were obtained from walnut trees grown in controlled soil and sand cultures, and also from trees in affected walnut groves. Comparisons have been made in some cases with results obtained from various species of fruit trees which had grown under similar conditions of exposure to saline soil.² More questions have been raised than have been answered and further studies are to be desired.

Very little has thus far been done in gaining an understanding of

¹ Paper no. 193, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² Through the kindness of Mr. RALPH LARUE, then of the field department of the California Walnut Growers Association, the writer has been brought into contact with the effects of saline soil conditions upon walnut trees in the field.

the effects of salts on trees, and the present preliminary study upon walnut trees is concerned with only one phase, that of chemical composition and behavior of the trees.

Method for large tank cultures

At the outset of this investigation, controlled experiments were carried on with walnut trees in soil in large galvanized iron tanks. The containers were cylindrical in shape and were 44 inches in diameter and 52 inches deep. The bottom of each tank tapered toward the center, where a heavy perforated brass plate covered the opening leading into a 2-inch drainage pipe. The surfaces of the tanks were heavily coated with asphalt paint, and when dry the tanks were placed in the soil to within 6 inches of the top of the tanks. Some finely crushed granite was placed in the bottom of each to promote drainage, and then soil was placed in each tank and firmly tamped as the tanks were being filled. A Placentia walnut tree budded on black walnut stock was planted in each tank in March, 1920. Poultry netting, 2 feet high and $\frac{3}{4}$ -inch mesh, with the upper edge bent down and outward, was placed about the upper rim of each tank as a protection against rodents, etc., and the treatment was begun.

In the first experiments, large quantities of soil were transported from the field and placed in the tanks in the same order, so far as possible, as the soil layers existed in the field. In no case could tree behavior be obtained, by growing walnut trees in these tanks, that was at all similar to that of the trees growing on this same soil in the groves from which the soil was obtained. Also it was found in preliminary studies that soils, obtained from walnut groves where poor tree growth prevailed, were distinctly improved for tree growth upon being transported to large tanks. The biological oxidation, the redistribution and reduction of localized high salt concentrations, and other factors no doubt play a large part in such soil improvement.

It was found that the heavy types of soil were not well adapted to tank culture work on account of the enormous shrinkage from the inner surface of the tanks and the tendency of such soils to produce large cracks as they dried somewhat. This made it extremely difficult to apply solutions to the soil and have the soil retain them

instead of their running directly into the drainage system. It was found that Ramona loam from the Box Springs tract of the Citrus Experiment Station and Sierra loam from the Rubidoux tract were both very satisfactory for tank cultures.

Six tanks were filled with Ramona loam soil taken from near the Box Springs Station tract, and a budded walnut tree was planted in each on March 30, 1920. A water extract of the soil used in filling the tanks gave the following results in terms of parts per million of dry soil: CO_3 , 0; HCO_3 , 183; NO_3 , 0; Cl , 18; SO_4 , 22; PO_4 , 18; total solids, 400.

TREATMENT OF TANK CULTURES

Three of the tanks (13, 14, and 15) received tap water having a pH of 7.7, while three of them (16, 17, and 18) received a synthetic

TABLE I
COMPOSITION OF IRRIGATION WATER (PARTS PER MILLION)

| | NA | CA | Mg | CO_3 | HCO_3 | NO_3 | Cl | SO_4 | TOTAL SOLIDS |
|---|-----|-----|-----|---------------|----------------|---------------|-----|---------------|--------------|
| Irrigation water used in unthrifty walnut grove..... | 221 | 128 | 163 | 0 | 308 | 10 | 240 | 759 | 1927 |
| Irrigation water (used in tanks 16-18) made artificially to imitate that used in unthrifty grove..... | 259 | 153 | 158 | 0 | 538 | 12 | 253 | 756 | 2108 |
| Tap water*..... | 89 | 50 | 18 | 0 | 236 | ... | 43 | 61 | 408 |

* Analysis of tap water January 26, 1916, by the Department of Chemistry, Citrus Experiment Station.

irrigation water made by adding salt solutions and tap water together so as to secure a certain ion concentration. This synthetic irrigation water (pH 7.8) was made to imitate the composition of water used to irrigate a soil near Whittier, California, on which walnut trees were most unthrifty.

Table I gives the ion concentration of the synthetic irrigation water given the soil in tanks 16-18. Each of the six tanks received at intervals the same amount of Hoagland's nutrient solution. It was observed that the water on the soil in tanks 16-18 had a greater rate of percolation than that of the control tanks 13-15, due no doubt to the flocculation brought about by the increased concentration of bases in the soil solution.

In July, 1920, the soil of all of the tanks was sampled and analyzed, and in table II are shown results typical of the two series. The use of this irrigation water from April to July brought about considerable increases in the water-soluble salts.

After the initial wetting of the soil in each tank with tap water over a period from March, 1920, to October, 1925, approximately 4500 liters of tap water and 2250 liters of Hoagland's solution made up with the tap water were added to each of tanks 13-15, and about

TABLE II
COMPOSITION OF A ONE-TO-FIVE WATER EXTRACT OF SOIL IN TERMS
OF PARTS PER MILLION OF DRY SOIL

| | AVERAGE OF TANKS 13-15 (CONTROL) | | | | AVERAGE OF TANKS 16-18 RECEIVING SALINE IRRIGATION WATER | | | |
|--------------------------------|-------------------------------------|-----|-----|-----|---|-----|-----|-----|
| | Depth in feet | | | | | | | |
| | 1 | 2 | 3 | 4 | 1 | 2 | 3 | 4 |
| Na..... | 30 | 16 | 19 | 10 | 66 | 20 | 39 | 21 |
| Ca..... | 21 | 23 | 19 | 20 | 62 | 35 | 50 | 27 |
| Mg..... | 9 | 8 | 8 | 6 | 21 | 12 | 18 | 9 |
| Cl..... | 23 | 27 | 21 | 21 | 71 | 41 | 44 | 27 |
| HCO ₃ | 82 | 70 | 92 | 73 | 76 | 76 | 76 | 92 |
| NO ₃ | 24 | 13 | 20 | 16 | 31 | 22 | 12 | 9 |
| SO ₄ | 29 | 22 | 6 | 1 | 18 | 53 | 123 | 27 |
| SiO ₂ | 35 | 30 | 20 | 26 | 28 | 41 | 35 | 33 |
| Total solids as sulphates..... | 208 | 169 | 164 | 128 | 518 | 241 | 378 | 203 |
| pH..... | 6.6 | 6.5 | 6.5 | 6.5 | 6.5 | 6.5 | 6.5 | 6.6 |

9400 liters of the synthetic irrigation water and 2250 liters of Hoagland's solution were added to each of tanks 16-18. The plan was to give each of tanks 13-15 tap water and occasionally Hoagland's solution, and when this was done, a like amount of Hoagland's solution was always at once added to each of tanks 16-18, plus additions of synthetic irrigation water.

DRAINAGE WATER FROM TANKS

The average results of analyses of the drainage water from tanks 13-15 and 16-18 are given in table III. They show the concentration of the various ions in the drainage water, and in particular the increasing concentration of total solids with time when a saline irrigation water is used. HEADLEY (3) has shown that growth is more

closely related to the proportion of salts recoverable from the soils than to the proportion of salts added.

GROWTH UNDER SALINE CONDITIONS

Fig. 1 shows the effect of the saline irrigation water on the total growth during 1921 of the walnut trees in tanks of soil. The curves each represent the average total growth of the shoots of three walnut trees in tanks of soil. Curves *B* and *C* are for trees in Yolo clay loam

TABLE III
COMPOSITION OF DRAINAGE WATER IN TERMS OF PARTS PER
MILLION OF SOLUTION

| | 3/30-4/30/1920 | | 5/1-6/21/1920 | | 6/22-9/25/1920 | | 10/1/1921 | | 10/1925 | |
|---------------------------------------|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|-------------------------|---|
| | 13-15 (CON- TROL) | 16-18 (SALINE IRRIGA- TION WATER) |
| Na..... | 13 | 58 | 82 | 74 | 57 | 20 | 71 | 190 | 243 | 2333 |
| K..... | | | | | | | | | 41 | 247 |
| Ca..... | 73 | 95 | 122 | 103 | 159 | 389 | 96 | 927 | 77 | 1374 |
| Mg..... | 47 | 22 | 16 | 21 | 39 | 122 | 32 | 291 | 50 | 652 |
| Cl..... | 22 | 19 | 20 | 23 | 67 | 358 | 66 | 1274 | 241 | 2957 |
| HCO ₃ | 311 | 356 | 476 | 440 | 482 | 578 | 311 | 336 | | |
| NO ₃ | 6 | 98 | 71 | 35 | 152 | 124 | 89 | 216 | | |
| SO ₄ | 58 | 54 | 69 | 78 | 64 | 417 | 71 | 1501 | 344 | 5252 |
| PO ₄ | | | | | 3 | 1 | | | | |
| Total solids as sul- phates.... | 666 | 553 | 729 | 631 | 1034 | 2808 | 702 | 5280 | 815 | 13150 |
| pH..... | 8.2 | 8.2 | 7.6 | 8.1 | 7.0 | 7.7 | 7.8 | 7.8 | | |

soil secured at two different locations near Whittier, California, and used in tank cultures; curve *A* represents the control trees (13-15); curve *D* represents the trees (16-18) in soil that received saline irrigation water. In the course of less than two years' use of the saline irrigation water, the soil solution seems to have somewhat reduced the growth rate.

The increase in the concentration of water-soluble salts in the soil receiving irrigation water brought about certain effects upon the walnut trees. According to the quantities of solution applied to the soil, trees 16-18 must have been less economical of the water supplied in relation to the growth produced. By the use of cobalt chloride

paper, a large number of transpiration tests were carried out upon the leaves of trees both in the control series and in that receiving saline irrigation water. Such rough tests indicated that the evapora-

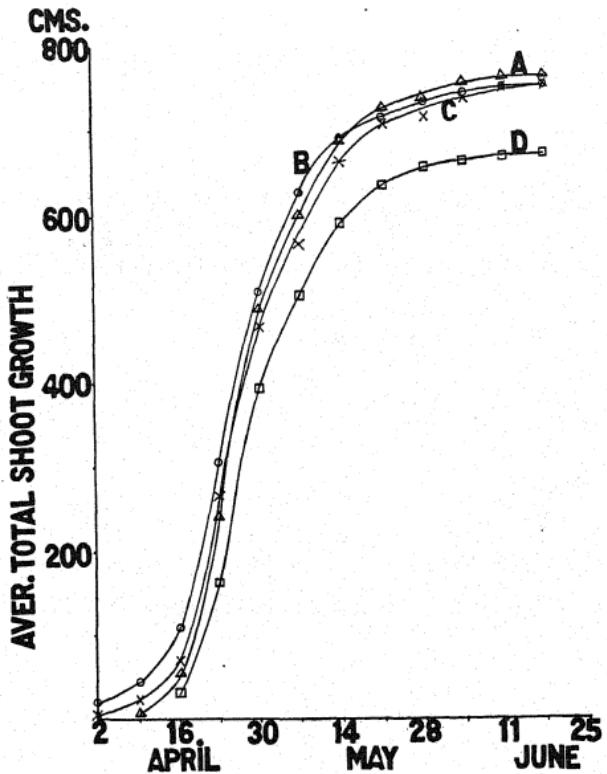


FIG. 1.—Effect of saline irrigation water on average total shoot growth of walnut trees: curves *B* and *C* represent average for trees in two different lots of Yolo clay loam soil obtained near Whittier, California; curves *A* and *D* are for trees in Ramona loam soil from Riverside, California. Hoagland's solution and tap water used for all trees; in addition, trees represented by curve *D* were given saline irrigation water.

tion rate from the leaves of the control trees was slightly less than, or equal to, that from the surface of a moist blotter; but that the evaporation rate from the leaves of the trees in soil receiving saline

irrigation water was somewhat greater than that from a moist blotter.

In October, 1925, the experiment was terminated. The root systems of trees 16-18, in soil irrigated with saline water, were much reduced, weighing an average of 13 pounds each; while those of the control trees 13-15 weighed an average of 20 pounds each. The average circumference 3 inches above the bud union was 20.1 inches for

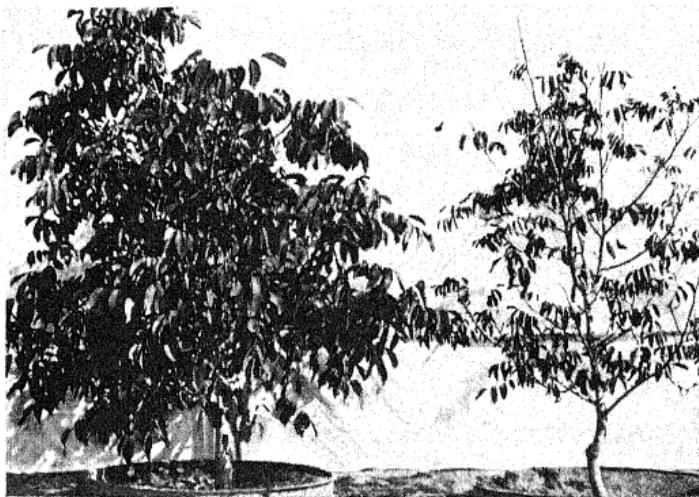


FIG. 2.—Effect of saline irrigation water on growth of walnut trees: left, tree 15, control in soil receiving Hoagland's solution and tap water; right, tree 16, in soil receiving Hoagland's solution and synthetic saline irrigation water.

trees 13-15 and 15.9 for trees 16-18. The average weight of the tops without the leaves for trees 13-15 was 14 pounds and for trees 16-18 was 6 pounds.

The saline irrigation water had an effect upon the size of walnut leaves, as shown in table IV. It was found that the leaves of trees in soil receiving saline water were smaller on successive cycles of growth. No mottling or "walnut yellows" was present. Slight burning of the leaves of trees 16-18 was seen during the last year of the experiment. Fig. 2 shows the dead shoots and the sparse reduced-

sized foliage of tree 16 as compared with tree 15; also the smaller diameter of the trunk about the bud union is clearly evident.

DISTRIBUTION OF INORGANIC CONSTITUENTS OF WALNUT SHOOTS AT END OF GROWING SEASON

Very little is known in regard to the effect of age on the composition of matured cycles of growth of walnut shoots. To gain such knowledge, composite samples of the cycles of growth for each group

TABLE IV

EFFECT OF SALINE IRRIGATION WATER UPON SIZE OF WALNUT LEAVES

SIZE OF AVERAGE LEAFLETS OF 25 WHOLE WALNUT LEAVES TAKEN JULY 29, 1925, FROM CONTROL TREES 13-15*

| No. measured to obtain size of average leaflet | Arrangement and designation | Designation | Average length (cm.) | Average width (cm.) |
|--|-----------------------------|-------------|----------------------|---------------------|
| 23 | I | I | 12.6 | 6.1 |
| 25 | | 2 | 12.9 | 5.7 |
| 25 | 2-3 | 3 | 12.9 | 5.6 |
| 25 | | 4 | 12.9 | 5.7 |
| 25 | 4-5 | 5 | 13.0 | 5.8 |
| 24 | | 6 | 10.6 | 4.9 |
| 23 | 6-7 | 7 | 10.6 | 5.0 |
| 18 | | 8 | 7.5 | 4.1 |
| 20 | 8-9 | 9 | 7.9 | 3.8 |
| 1 | | 10 | 8.0 | 4.0 |
| 1 | 10-II | II | 8.0 | 4.0 |

SIZE OF AVERAGE LEAFLETS OF 25 WHOLE WALNUT LEAVES TAKEN JULY 29, 1925, FROM TREES 16-18 THAT RECEIVED SALINE IRRIGATION WATER†

| 25 | I | I | 7.3 | 2.9 |
|----|-----|---|-----|-----|
| 25 | | 2 | 7.7 | 3.0 |
| 24 | 2-3 | 3 | 7.8 | 3.3 |
| 25 | | 4 | 7.7 | 3.3 |
| 24 | 4-5 | 5 | 7.6 | 3.3 |
| 24 | | 6 | 5.4 | 2.6 |
| 24 | 6-7 | 7 | 5.4 | 2.6 |
| 10 | | 8 | 4.7 | 2.1 |
| 10 | 8-9 | 9 | 4.5 | 2.3 |

* Average length of leaf stalk (25 whole leaves) 26.3 cm.

† Average length of leaf stalk (25 whole leaves) 14.4 cm.

of trees were obtained by pruning off the cycles in the order of their occurrence, from the shoot tips toward the base of the trunks. The growth of the shoots of walnut trees 13-15 and 16-18 usually consisted of only one cycle a year. An analysis of the samples is given in table V. It will be seen that, with the exception of the youngest cycles of growth, the ash as a percentage of the dry matter in trees 13-15 decreases from the youngest growth down to the coarse root-

lets. A similar condition exists in the samples from trees 16-18, except that here the decrease takes place in both directions, from the young shoots toward the root and from the coarser rootlets toward the trunk, with a minimum near the bud union or ground level. The roots of trees 16-18 were bathed by a soil solution extremely concentrated as regards certain salts. The gradient in the ash content is not so easily followed when the salts of the ash are studied,

TABLE V
EFFECT OF AGE AND SALINE SOIL ON COMPOSITION OF WALNUT SHOOTS

| | NOVEMBER 13, 1925 | ASH AS PER- CENTAGE DRY MATTER | PERCENTAGE DRY MATTER | | | | | | |
|--|---------------------|--------------------------------------|-----------------------|----------------------------------|----------------------------------|-------|-------|-------|-------|
| | | | Total Cl | Total S as SO ₄ | Total P as PO ₄ | Ca | Mg | Na | K |
| Trees 13-15, in soil re- ceiving good irriga- tion water | Apical (last) cycle | 7.42 | 0.07 | 0.28 | 1.56 | 2.13 | 0.39 | 0.04 | 0.59 |
| | Seventh cycle | 6.55 | 0.03 | .18 | 0.74 | | | | |
| | Sixth cycle | 7.07 | 0.04 | .19 | | 2.38 | .21 | .06 | .39 |
| | Fifth cycle | 6.83 | 0.02 | .14 | .70 | | | | |
| | Fourth cycle | 6.41 | 0.02 | .17 | .70 | 2.17 | .20 | .11 | .33 |
| | Third cycle | 5.97 | 0.02 | .13 | .81 | 1.96 | .20 | .16 | .35 |
| | Second cycle | 5.90 | 0.01 | .12 | .82 | | | | |
| | First cycle | 4.71 | | .25 | .67 | 1.44 | .22 | .17 | .27 |
| | Main root | 4.53 | | | | 0.92 | .17 | .13 | .72 |
| | Rootlets | 4.50 | 0.03 | .12 | 0.64 | 1.27 | .10 | .10 | .51 |
| Trees 16-18, in soil re- ceiving sa- line irriga- tion water | Apical (last) cycle | 6.30 | .49 | .74 | 1.14 | 1.20 | .80 | .17 | .54 |
| | Sixth cycle | 4.75 | .34 | .52 | 0.70 | | | | |
| | Fifth cycle | 7.76 | | .39 | .98 | 2.34 | .39 | .11 | .29 |
| | Fourth cycle | 6.15 | | .42 | .98 | | | | |
| | Third cycle | 5.64 | 0.20 | .32 | .96 | 1.72 | .31 | .15 | .28 |
| | Second cycle | 5.14 | | | | | | | |
| | First cycle | 4.90 | | | | 1.48 | .29 | .27 | .20 |
| | Main root | 5.07 | | | | 0.77 | .32 | 0.35 | .64 |
| | Rootlets | 6.61 | 1.19 | 0.44 | 0.57 | 1.22 | 0.22 | 1.00 | 0.38 |

for some of the constituents of the ash or dry matter are decreasing while others are increasing. The high values of the magnesium, total chlorine, sulphur, and phosphorus in trees 16-18 are outstanding. The gradients observed are indicative of the interdependence of the nutrition of one cycle of the tree on those that have preceded. The importance of this fact is often overlooked in reclamation studies upon salt-affected trees, the recovery of which is looked for at too early a date, long before the external and internal equilibria have had time for sufficient change to take place and reflect itself in improved appearance of the trees.

INORGANIC COMPOSITION OF WOODY SHOOTS IN
SPRING, AND CHANGES DUE TO GROWTH

The results given in table VI make clearer the effect of growth under normal conditions upon the composition of the tree, and the effect of the addition of a new cycle upon the composition of the

TABLE VI
EFFECT OF GROWTH UPON COMPOSITION OF MATURE PRIOR CYCLES
OF WALNUT SHOOTS FROM HEALTHY TREE

| | MATURE LAST CYCLE OF GROWTH OF 1925, REMOVED FOR ANALYSIS MAY 4, 1926, BEFORE NEW GROWTH HAD BEGUN | | | LAST CYCLE OF GROWTH OF 1925, REMOVED FOR ANALYSIS JUNE 15, 1926, AFTER NEW CYCLE HAD BEEN MADE FROM TERMINAL BUD AND HAD CEASED ELONGATING | | |
|--|---|-------------------------------------|-----------------|--|-------------------------------------|-----------------|
| | Soluble fraction (per cent) | Insoluble fraction (per cent) | Percent- age | Soluble fraction (per cent) | Insoluble fraction (per cent) | Percent- age |
| Ash as percentage of dry matter. | 1.98 | 8.88 | | 1.36 | 6.80 | |
| Ash of soluble fraction as percentage of total ash. | 18.22 | | | 16.63 | | |
| Total Ca as percentage of total ash. | | | 33.50 | | | 34.37 |
| Percentage of total Ca in each fraction. | 8.12 | 91.88 | | 6.28 | 93.72 | |
| Ca of each fraction as percentage of ash of same fraction. | 14.95 | 37.70 | | 12.98 | 38.64 | |
| Percentage of total Mg in each fraction. | 61.36 | 38.64 | | 42.48 | 57.52 | |
| Total Mg as percentage of total ash. | | | 4.05 | | | 2.37 |
| Percentage of total Na in each fraction. | 65.18 | 34.82 | | 53.70 | 46.30 | |
| Total Na as percentage of total ash. | | | 2.28 | | | 2.65 |
| Percentage of total K in each fraction. | 87.39 | 12.61 | | 91.38 | 8.62 | |
| Total K as percentage of total ash. | | | 3.20 | | | 5.41 |
| Total P as percentage of dry matter. | 0.23 | 0.21 | | 0.13 | 0.11 | |

cycle from which it took origin. In this table composite samples consisting of twenty-five shoots of a given cycle from a healthy walnut tree on the Rubidoux tract were used. After a new cycle is produced, the ash of both the water-soluble and water-insoluble fractions is reduced. Practically no change takes place in the total calcium as percentage of total ash, or in the general relationship involving calcium. The percentage of total magnesium in the ash of a cycle is reduced after a new cycle has grown from it, and this is largely repre-

sented by the migration of water-soluble magnesium. The total potassium as a percentage of the ash is increased after new growth is produced. A decided loss of total phosphorus takes place in both fractions of the dry matter of a shoot when new growth is produced from it. It is evident that the growth of one portion of the tree brings about changes in the composition of older portions, and brings about changes in the equilibrium conditions within the tree and no doubt also in the soil solution.

EFFECT OF SALINE IRRIGATION WATER ON COMPOSITION OF WALNUT LEAVES FROM TREES IN TANK CULTURES

The saline condition of the soil is reflected not alone in the composition of the woody growth cycles that compose the shoots (table V), but also in the marked changes that take place in the composition of the leaves. Samples of leaves of the same age were taken from the trees of both series, 13-15 and 16-18, and analyses were made of their ash (table VII). The ash of the walnut leaves from trees 16-18, when calculated as a percentage of the dry matter, is always lower than that of the control trees 13-15, from which one may infer that the organic matter of the dry leaves from trees 16-18 was greater than that of the leaves from the control trees 13-15. This was at least expected on account of the high concentration of the soil solution in tanks 16-18, and is therefore of unusual interest. The amount of sodium present in the leaves of both series is not very large, when we consider that sodium was the base applied in the largest amount in the irrigation water. The amount of potassium in the dry matter of the leaves of trees from tanks 13-15 is always larger than that of leaves from the trees in tanks 16-18 that were irrigated with saline water free from potassium. Table III shows that in October, 1925, the drainage water from tanks 16-18 contained very much larger concentrations of sodium and potassium than that from tanks 13-15, and yet the leaves from trees 16-18 contained less potassium than those from trees 13-15. This is no doubt due in part to the reduced ash content of the leaves from trees 16-18, and in part to the small amount of potassium present in the soil solution, as indicated by the drainage water in comparison with the large concentration of other bases. It will be observed that near the start of the experiment

the potassium present in the leaves of trees 16-18 approached that present in the leaves of trees 13-15, but that it fell off considerably as the trees became affected by the saline irrigation water.

It is of interest that the calcium content of the dry matter of the leaves of trees 13-15 is always greater than that of trees 16-18. A reduced calcium content is also typical of walnut leaves affected with "yellows" (HAAS, BATCHELOR, and THOMAS 2), the symptoms of

TABLE VII
EFFECT OF SALINE IRRIGATION WATER UPON ASH COMPOSITION
OF WALNUT LEAVES

| TREES* | DATE OF SAMPLING | ASH AS PERCENT- AGE OF DRY MATTER | GRAMS IN 100 GM. OF DRY MATTER (CALCULATED FROM ASH ANALYSES) | | | | | | |
|--------------|------------------|---|--|-------|-------|-------|-------|-----------------|-----------------|
| | | | Na | K | Ca | Mg | Cl | SO ₄ | PO ₄ |
| Control..... | Nov. 2, 1922 | 9.29 | 0.250 | 1.095 | 2.035 | 0.430 | 0.137 | 0.206 | 0.602 |
| Treated..... | Nov. 8, 1922 | 8.64 | .284 | 1.063 | 1.620 | .626 | .698 | .208 | .444 |
| Control..... | July 28, 1923 | 9.84 | .367 | 1.635 | 1.932 | .458 | .110 | .272 | .678 |
| Treated..... | July 28, 1923 | 7.17 | .362 | 1.387 | 1.001 | .622 | .484 | .284 | .529 |
| Control..... | Nov. 15, 1923 | 9.77 | .285 | 1.192 | 2.228 | .420 | .188 | .165 | .794 |
| Treated..... | Nov. 15, 1923 | 6.87 | .078 | 0.508 | 1.370 | .708 | .517 | .177 | .491 |
| Control..... | Dec. 3, 1924 | 10.13 | | | 2.247 | .492 | .366 | .270 | .649 |
| Control..... | Nov. 19, 1924 | 9.49 | .313 | 1.125 | 1.929 | 0.410 | .259 | .241 | .742 |
| Treated..... | Nov. 19, 1924 | 8.52 | .202 | 0.667 | 1.320 | 1.126 | .943 | .405 | 0.491 |
| Control..... | July 29, 1925 | 8.28 | .337 | 1.295 | 1.534 | 0.429 | .106 | .207 | |
| Treated..... | July 29, 1925 | 6.90 | 0.249 | 0.880 | 0.976 | 0.880 | 0.747 | 0.338 | |

* Control, trees 13-15, Hoagland's solution with tap water; treated, trees 16-18, same with added salts.

which were absent from these trees. In walnut leaves affected with yellows, besides a reduced calcium content there is an increased potassium content, a condition which was not found in trees 16-18. It seems, therefore, that this is further evidence, although indirect, that walnut leaves affected with yellows are characterized not only by a reduced calcium content but also by an increased potassium content.

The magnesium content of the ash of the leaves of trees 16-18, in soil receiving saline irrigation water, was considerably higher than that of trees 13-15; and in the last two samples of leaves from trees 16-18 the content of magnesium approached that of calcium.

The large concentration of chlorine in the drainage water (table III) was accompanied by increased concentrations of inorganic

chlorine in the leaves of trees 16-18. The concentration of sulphate in the drainage water also was large, and in fact usually exceeded that of chlorine. In the first three samplings of leaves from trees 16-18, however, the increases in the sulphate content of the ash over that of leaves from trees 13-15 were extremely small. The phosphate content of the ash, when calculated as a percentage of the dry matter, is much reduced in the leaves of trees 16-18 below that of leaves from trees 13-15. The organic chlorine, sulphur, and phosphorus in walnut and other tree leaves will be referred to later.

The effect of irrigation water rich in sodium, calcium, magnesium, chlorine, and sulphate upon the ash composition of the leaves of the experimental trees is a reduction in the content of calcium and an increase in the content of chlorine and sulphate. It is of interest that the sodium, potassium, and calcium contents of the affected leaves are actually reduced while that of magnesium is increased. In table VII is seen the great uniformity that exists in the concentration of magnesium in the dry matter of the leaves taken from the control trees 13-15, and the great variation in that of the leaves taken from trees 16-18.

EFFECT OF SALINE IRRIGATION WATER ON COMPOSITION OF WALNUT FRUITS FROM TREES IN TANK CULTURES

A comparison of the analyses of the ash of walnut husks, shells, and kernels from both sets of trees is given in table VIII. The ash of the husks as a percentage of the dry matter is least in the case of the trees that grew in soil receiving saline irrigation water, which is not what one would expect to find. The husks of trees 16-18 contain less sodium, potassium, and calcium, but more magnesium, chlorine, and sulphate than those of trees 13-15. The same relationship holds for the composition of the shells as for the husks, except in the case of sulphur. The dry matter of the kernels from nuts of trees 16-18 in 1923 contains less ash, and the ash contains less potassium, calcium, and phosphorus than in the case of trees 13-15. In 1923 the kernels from trees in both series were plump and uniform. In 1925 the kernels from trees 13-15 were plump, however, while those from trees 16-18 were somewhat shriveled and dark brown. The shriveling and discoloration explain the fact that the ash constituents

of the kernels (on the basis of dry matter) from trees 16-18 exceed in every case those of the kernels from trees 13-15; the ash is increased while the dry matter is decreased. A more detailed investigation of the shriveling of walnut kernels has been made by HAAS and BATCHELOR (1).

Attention should be called to the striking situation that exists in walnut husks. The husks are green, and very likely take part in

TABLE VIII

EFFECT OF SALINE IRRIGATION WATER UPON ASH COMPOSITION OF WALNUT
HUSKS, SHELLS, AND KERNELS

| MATERIAL | TREES* | DATE OF SAMPLING | ASH AS PERCENTAGE OF DRY MATTER | GRAMS IN 100 GM. OF DRY MATTER (CALCULATED FROM ASH ANALYSES) | | | | | | |
|------------|---------|------------------|---------------------------------|---|---------|--------|--------|--------|-----------------|--------|
| | | | | Na | K | Ca | Mg | Cl | SO ₄ | |
| Husks... | Control | Oct. 1923 | 14.60 | 1.6575 | 6.46150 | 0.7340 | 0.1410 | 0.0910 | 0.3250 | 0.497 |
| | Treated | | 12.28 | 1.4345 | 5.4315 | .459 | .304 | .420 | .446 | .295 |
| | Control | Sept. 9, 1925 | 12.08 | 1.635 | 6.044 | .354 | .104 | .085 | .336 | .615 |
| | Treated | | 9.30 | 1.151 | 4.518 | .156 | .245 | .765 | .521 | .722 |
| Shells... | Control | Oct. 1923 | 1.68 | 0.130 | 0.425 | .284 | .039 | .007 | .016 | .045 |
| | Treated | | 1.20 | .055 | .329 | .210 | .040 | .011 | .014 | .007 |
| | Treated | Nov. 6, 1924 | 1.25 | .083 | .304 | .145 | .051 | .021 | .013 | .047 |
| | Control | | 2.03 | .121 | .487 | .102 | .176 | .005 | .020 | .1.271 |
| Kernels... | Control | Oct. 1923 | 2.12 | .107 | .482 | .096 | .169 | .004 | .010 | .349 |
| | Treated | | 1.68 | .091 | .418 | .069 | .164 | .004 | .025 | |
| | Treated | | 1.74 | .114 | .415 | .083 | .165 | .005 | .015 | .084 |
| | Control | Sept. 9, 1925 | 2.31 | .060 | .480 | .124 | .184 | .004 | .016 | .006 |
| | Treated | | 3.45 | 0.140 | 0.820 | 0.2370 | 0.2110 | 0.0280 | 0.063 | 1.159 |

* Control, trees 13-15, Hoagland's solution with tap water; treated, trees 16-18, same with added salts.

photosynthetic processes to some extent, like leaves. The dry matter of the husks of trees 13-15, however, contains about 1.6 per cent of sodium and about 6 per cent or more of potassium. The calcium concentration in the dry matter of the husks is very low in comparison with that of sodium and potassium. The ash content of the dry matter of the husks usually greatly exceeds that of the dry matter of the leaves. In the dry matter of good walnut leaves (table VII) the concentration of calcium is much greater than that of potassium while that of sodium is very small. If the husks therefore possess photosynthetic systems near their peripheral surface only, then either the large concentrations of sodium or potassium are only in deeper layers, or else such a photosynthetic system can function in

the presence of large concentrations of salts. Later tables consider these salts of the walnut husk, their solubility, the influence of sunlight on their composition, their organic combination, and other factors of interest in this connection.

EFFECT OF SHADING ON COMPOSITION OF WALNUT FRUITS

The effect of sunlight on the ash composition of walnut husks and kernels is seen in table IX. Some of the nuts attached to the tree were covered when about six weeks old with heavy brown paper bags, which excluded much but not all of the light. Both the control and the bagged walnuts were on the same tree at Irvine, California, and were removed on September 18. Table IX shows that the dry matter of the husks in full sunlight contained more ash than when given partial sunlight. It is not unlikely that this increase of ash in the husks of walnuts exposed to full sunlight may become so great in the interior districts of California, where high temperatures in late summer may prevail, that injurious concentrations of ash may bring about the burning, to the point of destruction or at least unmarketability of the nut. The percentages of sodium, potassium, and phosphorus in the ash were somewhat less, but the percentage of calcium was greater when the husks were given full than when given partial sunlight. The dry matter of the husks showed a greater concentration of all the determined ash constituents, except phosphorus, when in full than when in partial sunlight, possibly due to a great transpiration rate. The sunlight had but little effect on the total ash content of the dry kernels. Those from the walnuts in full sunlight had the greater percentages of sodium, potassium, and calcium, whether calculated in terms of the ash or of the dry matter. The husks and their kernels both contained less calcium when given partial sunlight than when given full sunlight. IRVING and BECKING (5) have shown that corallines use up more calcium in the light than in darkness. It is of interest to find (table IX) that, accompanying the reduced calcium in the kernels from nuts given partial sunlight, there is a considerable increase in the concentration of magnesium. It is also of interest that the sum of the magnesium of the husks and kernels in 100 gm. dry matter is about the same after exposure to full sunlight as after exposure to partial sunlight only. This type of relation-

ship does not hold for any of the other bases. It might be construed to mean that less magnesium is required in partial sunlight by the photosynthetic processes, the kernel appropriating the portion not required by the husk. It also indicates that magnesium absorption may be independent of the action of full sunlight, since the total magnesium is about the same in the two cases.

TABLE IX
EFFECT OF SHADING ON ASH COMPOSITION OF WALNUT HUSKS AND KERNELS
WHILE ATTACHED TO TREE

| | EXPOSED TO SUNLIGHT | | EXPOSED TO SUNLIGHT UNTIL ABOUT SIX WEEKS OLD, THEN COVERED WITH HEAVY BROWN PAPER BAGS | |
|--------------------------------------|---------------------|---------|--|---------|
| | Husks | Kernels | Husks | Kernels |
| Ash as percentage of dry matter..... | 22.11 | 2.75 | 18.19 | 2.82 |
| Ash constituents (percentage of ash) | | | | |
| Na..... | 10.12 | 2.86 | 11.94 | 1.57 |
| K..... | 44.65 | 22.09 | 47.47 | 17.24 |
| Ca..... | 3.89 | 4.42 | 2.49 | 3.10 |
| Mg..... | 1.11 | 3.16 | 0.83 | 6.83 |
| Cl..... | 13.71 | 1.29 | 12.53 | 1.28 |
| SO ₄ | 1.46 | 0.64 | 1.59 | 0.40 |
| PO ₄ | 3.57 | 40.28 | 5.39 | 39.40 |

Grams in 100 gm. of dry matter (from ash analyses)

| | | | | |
|-----------------------|--------|--------|--------|--------|
| Na..... | 2.2375 | 0.0787 | 2.1719 | 0.0443 |
| K..... | 9.8721 | 0.6075 | 8.6348 | 0.4862 |
| Ca..... | 0.8601 | 0.1216 | 0.4529 | 0.0900 |
| Mg..... | 0.2454 | 0.0869 | 0.1510 | 0.1926 |
| Cl..... | 3.0313 | 0.0355 | 2.2792 | 0.0361 |
| SO ₄ | 0.3228 | 0.0170 | 0.2892 | 0.0113 |
| PO ₄ | 0.7893 | 1.1077 | 0.9804 | 1.1111 |

ASH OF WALNUT LEAVES AFFECTED BY ACCUMULATION OF CHLORINE

An examination of walnut trees under field conditions where the soil contains considerable alkali salts shows that considerable amounts of chlorine may be absorbed. When the accumulation of chlorine becomes excessive as the leaf matures, marginal and tip burning results and the leaves may fall prematurely. The trees in some walnut districts may have chlorine burn without other complicating factors, such as excessive concentration of various other alkali

salts. In these cases the soil may contain large amounts of soluble calcium, and under such conditions a given concentration of chlorine may be less injurious than when less favorable bases are in preponderance. Table X gives data in regard to the chlorine content of the ash of leaves obtained from walnut trees of this nature.

TABLE X
COMPOSITION OF ASH OF WALNUT LEAVES AFFECTED BY CHLORINE

| | LEAVES FROM TREES IN SALINE SOIL, IRVINE, CALIF., JUNE 5, 1925 | LEAVES FROM TREES SURROUNDED BY SALT-INJURED TREES, NO INJURY AS YET, IRVINE, JUNE 22, 1925 | SALTY SOIL NEAR DRAINAGE DITCH, LEAVES BURNED, IRVINE, JUNE 22, 1925 | SALT BURN, IRVINE, JULY 13, 1925 | LEAF BURN, IRVINE, SEPT. 18, 1925 | GOOD LEAVES, IRVINE, SEPT. 18, 1925 (1), GOOD LEAVES, ANAHEIM, CALIF., SEPT. 18, 1925 (2) | MARGINAL BURNING, VENTURA, CALIF., SEPT. 22, 1925 | MARGINAL BURNING, NEARLY ALL LEAVES PREMATURELY ABSICSED FOR TWO SUCCESSIVE SEASONS, SANTA BARBARA, CALIF., SEPT. 21, 1925 |
|--------------------------------------|---|---|--|----------------------------------|-----------------------------------|---|--|---|
| Ash as percentage of dry matter..... | 10.19 | 9.90 | 8.47 | 10.26 | 11.55 | 12.75(1) | 12.67 | 11.81 |
| Ash constituents (percentage of ash) | | | | | | | | |
| Na..... | 5.33 | 3.43 | 5.10 | 4.53 | 2.32 | 3.02(1) | 1.73 | 4.89 |
| K..... | 19.13 | 12.24 | 15.95 | 16.26 | 6.78 | 12.54(1) | 7.27 | 15.86 |
| Ca..... | 17.67 | 23.58 | 19.75 | 18.60 | 20.09 | 23.13(1) | 26.53 | 18.60 |
| Mg..... | 6.05 | 6.76 | 7.46 | 7.64 | 9.11 | 6.70(1) | 6.03 | 6.11 |
| Cl..... | 13.34 | 8.50 | 17.78 | 14.89 | 9.47 | 4.54(1) | 7.25 | 13.31 |
| | | | | | | 1.58(2) | | |
| SO ₄ | 2.42 | 2.10 | 3.28 | 2.57 | 2.73 | 2.42(1) | 2.79 | 4.41 |
| PO ₄ | 12.31 | 8.06 | 12.00 | 8.57 | 11.82 | 3.85(2) | 4.92 | 6.90 |
| | | | | | | 4.28(1) | | |

The sodium content of the ash of these leaves is never high, and the calcium content is like that of normal leaves of the same age. It may be noted that good walnut leaves collected as late as September 18 have been found to contain from 1.5 to 5 per cent of chlorine in their ash without visible injury, although the larger amount may not be conducive to the best growth the trees might make, were the chlorine content lower. Where the soil solution is rich in chlorine, walnut leaves may contain excessive amounts. The values given in table X represent only the non-volatile chlorine, and these alone are

extremely high in some cases. It will be seen that the sulphate content of the ash of walnut leaves in table X is very uniform, although many of the soils in which the trees were growing are moderately rich in sulphate as well as in chlorine. The sulphate of calcium is not as soluble as the chloride. The reason for the low concentration of sulphate, although not for its uniformity, is the fact that much of the sulphur in walnut leaves is present in the organic form. Reference will be made to the organic chlorine and sulphur in a later connection.

The sensitivity of walnut trees to salts is seen in experiments in which walnut trees were grown in soil in large pipes 5-6 feet high and about 2 feet in diameter. As the soil was being placed in the pipes, a layer of one of the calcium salts about 0.25 inch deep was placed in the soil about one-fourth of the distance down from the top of the soil. This was in imitation of strata of salts that may possibly occur in soils, since it is difficult otherwise to explain why trees may suddenly be injured, or may suddenly change from poor to good growth.

A single layer was made in each pipe, calcium hydrate, tricalcium phosphate, and calcium carbonate being used for different pipes. After making excellent growth early in the season, the trees began to show bad leaf burn without showing more calcium or magnesium in the leaves than occurs in good walnut leaves of the same age, while the trees in untreated soil showed excellent growth throughout the experiment. In such cases the salts probably act by hindering absorption of water by the roots, rather than by producing excessive salt concentrations in the leaves.

COMPARATIVE CHLORINE ACCUMULATIONS IN DECIDUOUS-LEAVED TREES

Other deciduous-leaved trees are also sensitive to chlorine accumulation, and it may be well to consider some others in comparison with the walnut. Table XI shows the increased amounts of chlorine in injured persimmon leaves collected in the autumn. The leaves were all mature, and instead of showing the autumnal colors typical of this species, they began to burn and absciss, and many of the fruits fell before they were ready to be picked. The chlorine in the

soil was usually accompanied by abundant calcium, and simply leaching the soil to carry down the excess chlorine was sufficient to bring the trees back to an excellent condition the following year. The persimmon trees were interplanted with grapefruit trees which showed no unfavorable symptoms. It is seen in table XI that about half of the chlorine, sulphur, and phosphorus exists in the organic form. Deciduous trees differ from citrus in their behavior as regards

TABLE XI
EFFECT OF ALKALI SALTS ON COMPOSITION OF PERSIMMON LEAVES

| | GOOD LEAVES, LA HABRA, CALIF. | GOOD LEAVES, TUSTIN, CALIF. | LEAVES JUST BE- GGINING TO SHOW DRIED SPOTS AND MARGINAL BURN, ORANGE | LEAVES BURNED ALONG MARGINS AND BETWEEN VEINS, ORANGE | BADLY BURNED LEAVES, ORANGE, CALIF. |
|--------------------------------------|-------------------------------------|-----------------------------------|---|--|---|
| Ash as percentage of dry matter..... | 14.86 | 15.43 | 16.17 | 14.88 | 16.53 |
| Ash constituents (percentage of ash) | | | | | |
| Na..... | 1.50 | 1.98 | 2.05 | 2.69 | 1.94 |
| K..... | 9.94 | 9.20 | 9.67 | 11.36 | 8.79 |
| Ca..... | 24.75 | 24.70 | 25.00 | 22.94 | 24.82 |
| Mg..... | 6.83 | 6.87 | 6.86 | 4.73 | 6.34 |
| Cl..... | 1.80 | 2.89 | 4.25 | 6.88 | 7.90 |
| SO ₄ | 2.52 | 3.56 | 2.61 | 3.47 | 2.81 |
| PO ₄ | 3.99 | 3.82 | 3.85 | 4.22 | 4.23 |
| Percentage of dry matter..... | | | | | |
| Total Cl..... | 0.54 | | | 1.70 | 2.22 |
| Cl of ash..... | 0.27 | | | 1.02 | 1.31 |
| Total S as SO ₄ | 0.71 | | | 0.95 | 0.88 |
| SO ₄ of ash..... | 0.38 | | | 0.52 | 0.47 |
| Total P as PO ₄ | 0.68 | | | | |
| PO ₄ of ash..... | 0.59 | | | | |

absorption of chlorine or sulphur, in that they lose much of their accumulation by means of leaf abscission each year, whereas citrus trees must go on adding to the accumulation over a period of years. The table shows the small percentage of sodium in the ash and the relatively large concentration of divalent in comparison with monovalent bases.

A comparison of the deciduous walnut tree with the evergreen citrus tree as regards the organic combination of the accumulated chlorine, sulphur, and phosphorus, is shown in table XII. When

several samples from trees have been taken in a given locality they are designated by numbers in the table. The ash of lemon fruit may contain all of the chlorine and much of the sulphur when the amount present is small, but when comparatively large amounts are present much may be in the organic form. Most of the phosphorus in lemon fruit is retained upon ashing at low temperatures. Much of the chlorine and sulphur of citrus leaves containing large amounts of these constituents is lost when the dry matter is ashed, presumably because they are in organic combination. Most of the sulphur of walnut shoots is in organic form, while the phosphorus is almost entirely retained in the ash. As much as one-half of the chlorine of walnut leaves may be lost in ashing the dry matter at low temperatures, while the organically combined sulphur and phosphorus may be considerable. In walnut leaves taken from sand cultures in which calcium sulphate was mixed with the sand, and the sand given Hoagland's solution lacking calcium, the dry matter of the leaves showed 2.40 per cent total S as SO_4 , while normal leaves contained 0.99 per cent. Practically all of the chlorine of walnut husks in table XIII is retained upon ashing the dry matter, as is most of the sulphur and phosphorus.

ABSORPTION FROM SOLUTIONS OF SODIUM CHLORIDE AND SODIUM SULPHATE BY GROWING FRUITS

If the shoots attached to walnuts are placed into solutions such as distilled water, N/100 sodium chloride, or N/100 sodium sulphate, and then the whole nuts, husks, and nuts with husk removed are analyzed, it is found that an appreciable gain in concentration in sodium has been made only in the nuts with husks removed (table XIII). In other words, the kernel gained largely in sodium content but the husk very little if at all. In the sodium chloride experiments practically no change took place in the chlorine content of the ash, but a gain in the total chlorine took place in the dry matter of the husks and kernels. The sulphur content of the ash of the husks greatly increased in the sodium sulphate solutions, although that of the kernels showed no appreciable increase. Sodium chloride solutions increased the organic chlorine in the husks and kernels, while sodium sulphate solutions increased the organic sulphur in both

TABLE XII
TOTAL CHLORINE, SULPHUR, AND PHOSPHORUS CONTENT OF DRY MATTER OF CITRUS AND WALNUT TREES COMPARED WITH CONTENT OF THESE CONSTITUENTS IN THE ASH

husks and kernels. Absorption of inorganic salts from such solutions by walnuts through attached shoots was accompanied by increases

TABLE XIII
EFFECT OF SALT SOLUTIONS ON COMPOSITION OF WALNUTS ON SHOOTS
PLACED IN SOLUTION

| SHOOTS 4"-6" LONG WITH TERMINAL NUTS; BASE OF SHOOT PLACED IN SOLUTION | DISTILLED WATER | | | N/100 NaCl | | | N/100 Na ₂ SO ₄ | | |
|---|--|-------|--------------------------------------|--|-------|--------------------------------------|--|-------|--------------------------------------|
| | Whole nuts (husk, shell, kernel) | Husks | Nuts with husk re- moved | Whole nuts (husk, shell, kernel) | Husks | Nuts with husk re- moved | Whole nuts (husk, shell, kernel) | Husks | Nuts with husk re- moved |
| Ash as percentage of dry matter... | 5.79 | 6.09 | 6.20 | 6.38 | 5.69 | 6.10 | 5.95 | 5.78 | 6.26 |
| Ash constituents (percentage of ash) | | | | | | | | | |
| Na..... | 7.22 | 9.09 | 6.40 | 7.00 | 9.73 | 13.26 | 9.34 | 8.06 | 10.30 |
| K..... | 37.49 | 30.15 | 40.89 | 33.35 | 33.21 | 39.43 | 35.86 | 28.35 | 40.87 |
| Ca..... | 9.32 | 11.80 | 5.39 | 10.29 | 9.45 | 4.96 | 8.14 | 11.30 | 5.25 |
| Mg..... | 3.21 | 3.78 | 4.43 | 3.90 | 3.67 | 3.90 | 3.38 | 4.08 | 4.16 |
| Cl..... | 1.93 | 1.63 | 2.66 | 2.01 | 1.92 | 2.92 | 1.62 | 1.52 | 3.03 |
| SO ₄ | 4.28 | 4.80 | 2.45 | 3.97 | 4.05 | 3.01 | 7.99 | 12.38 | 3.20 |
| PO ₄ | 14.89 | 11.70 | 19.94 | 13.01 | 11.85 | 18.67 | 15.20 | 11.14 | 16.96 |
| Percentage of dry matter | | | | | | | | | |
| Cl..... | 0.11 | 0.14 | | | 0.24 | 0.27 | | | |
| SO ₄ | 0.22 | 0.18 | | | | | 0.38 | 0.44 | |

TABLE XIV
EFFECT OF CHLORIDES UPON ASH COMPOSITION OF WALNUT HUSKS

| | GOOD WALNUT HUSKS, IRVINE, SEPT. 18, 1925 | WALNUT HUSKS FROM TREES ON ALKALI SOIL, IRVINE, SEPT. 18, 1925 | WALNUT HUSKS SPOTTED AND BURNED, SALT ON HUSKS, ALKALI SOIL, IRVINE, SEPT. 18, 1925 |
|--------------------------------------|---|--|--|
| Ash as percentage of dry matter..... | 22.99 | 20.62 | 16.75 |
| Ash constituents (percentage of ash) | | | |
| Na..... | 10.82 | 10.09 | 10.62 |
| K..... | 44.94 | 40.76 | 41.92 |
| Ca..... | 4.86 | 4.09 | 4.56 |
| Mg..... | 1.62 | 1.24 | 2.00 |
| Cl..... | 3.53 | 15.86 | 13.18 |
| SO ₄ | 2.46 | 2.37 | 5.61 |
| PO ₄ | 1.45 | 5.86 | 10.75 |

in chlorine or sulphur, some of which were almost entirely of organic nature. Such experiments were carried on with frequent changes of

solution during early June, when the kernel contents were still in a more or less liquid or partially jelled condition.

If the chlorine is allowed to accumulate in walnut husks slowly over a long period in the field, however, and the samples gathered for analysis when the monovalent and divalent bases of the husks are high, the ash of the husks may be found to contain large amounts of chlorine (table XIV). In contrast with the low amount of chlorine in the husks of good walnuts, walnut husks may accumulate so much



FIG. 3.—Walnuts of different ages showing extreme effects of chloride accumulation upon husks. Chemical tests of light-colored incrustations in sunken areas indicate accumulations of chloride.

chlorine that it can be gently scraped off the surface with a scalpel onto a slide (fig. 3), and the test for chlorine obtained with silver nitrate.

SODIUM AND POTASSIUM IN LEAVES AND FRUITS

If various tree materials are ashed in the presence of sulphuric acid, to prevent volatilization of the alkali bases, it is found that the bases of the dry matter are practically non-volatile (table XV). This is of great interest, in that table XVI shows the large concentration of bases of which potassium is outstanding in amount. The results given in table XVI represent the average of three closely agree-

ing analyses of six entire husks that had not touched the ground. It still remains to be determined whether the bases are combined with silicates, carbonates, bicarbonates, nitrates, etc., as the chlorine content of these husks is known to be low, and the reaction values of basic and acidic constituents do not approach a balance.

TABLE XV
COMPARISON OF TOTAL SODIUM AND POTASSIUM OF DRY MATTER WITH THAT
FOUND IN ASH OF TREES

| MATERIAL | No H_2SO_4 USED IN ASHING DRY MATTER | | H_2SO_4 USED IN ASHING DRY MATTER | |
|---|---|-------|--|-------|
| | Percentage of dry matter | | | |
| | Na | K | Na | K |
| Valencia orange leaves from trees in soil given excessive potassium..... | 0.14 | 5.90 | | 5.95 |
| Good Placentia walnut leaves..... | 0.07 | 1.33 | 0.07 | 1.34 |
| Good Placentia walnut husks..... | | 10.34 | | 10.54 |
| Good Placentia walnut kernels..... | 0.04 | 0.43 | 0.07 | 0.44 |
| Persimmon leaves..... | 0.10 | | 0.11 | |

TABLE XVI
COMPOSITION OF ASH OF EUREKA WALNUT HUSKS FROM TREES AT
BOX SPRINGS TRACT, OCTOBER 20, 1926

| | | ASH AS PERCENT- AGE OF DRY MATTER | PERCENTAGE OF ASH | | | | | |
|------------------------------------|---------------|---|-------------------|--------|--------|--------|--------|--------|
| | | | Na | K | Ca | Mg | SO_4 | PO_4 |
| Fresh weight | Dry weight | 15.82 | 10.69 | 43.08 | 6.88 | 1.66 | 1.97 | 4.00 |
| Grams per husk (from ash analyses) | | | | | | | | |
| 23.66 | 3.25 | 0.5136 | 0.0549 | 0.2211 | 0.0354 | 0.0086 | 0.0101 | 0.0205 |

SEASONAL CHANGES IN ASH CONSTITUENTS
OF YOUNG WALNUT FRUITS

If the composition of the ash of good walnut fruits of varying ages is studied (table XVII), it is found that the ash of the dry matter of the kernels decreases with age, because the increase in the weight of the dry matter is more rapid than that of the increase in

TABLE XVII
CHANGES IN COMPOSITION OF ASH OF GOOD WALNUT FRUITS OF DIFFERENT AGES

| Ash as percentage (percentage of ash) | MAY 26, 1925 | | JUNE 3, 1925 | | JUNE 9, 1925 | | JUNE 22, 1925 | | AUGUST 14, 1925 | |
|---|--------------|--|--------------|--|--------------|--|---------------|--|-----------------|--|
| | Husks | Whole nuts (husk, shell, kernel) | Husks | Whole nuts (husk, shell, kernel) | Husks | Whole nuts (husk, shell, kernel) | Husks | Whole nuts (husk, shell, kernel) | Husks | Whole nuts (husk, shell, kernel) |
| Ash as percentage (percentage of ash) | 7.03 | 6.68 | 6.33 | 6.18 | 5.86 | 6.09 | 6.05 | 5.49 | 5.47 | 6.70 |
| Na. | 8.11 | 8.63 | 11.28 | 7.55 | 9.76 | 9.63 | 8.65 | 9.53 | 12.63 | 10.85 |
| K. | 29.51 | 33.09 | 40.32 | 33.20 | 35.49 | 39.19 | 35.08 | 35.60 | 40.44 | 39.00 |
| Ca. | 13.10 | 10.30 | 4.32 | 10.19 | 8.42 | 10.07 | 8.24 | 4.93 | 7.34 | 7.18 |
| Mg. | 4.88 | 4.07 | 3.48 | 4.12 | 3.90 | 3.39 | 3.87 | 3.86 | 3.14 | 3.14 |
| Cl. | 2.08 | 1.84 | 1.45 | 2.28 | 1.71 | 1.81 | 1.99 | 1.58 | 1.98 | 1.59 |
| SO ₄ . | 5.86 | 4.53 | 3.50 | 4.11 | 2.77 | 5.31 | 4.50 | 3.01 | 4.70 | 3.67 |
| PO ₄ . | 11.47 | 15.52 | 19.81 | 9.18 | 13.62 | 19.95 | 9.46 | 13.84 | 20.61 | 8.47 |
| Fresh weight (gm.) | 228 | (25)235 | 217 | (25)496 | 542 | 269 | (25)621 | | (20)320 | (20)653 |
| Dry weight (gm.) | 32 | 30 | 21 | 75 | 59 | 49 | 41 | 76 | 42 | 97 |

| | | | | | | | | | | |
|-------------------------------|---|-------------------------------|---|-------------------------------|---|-------------------------------|---|-------------------------------|---|-------------------------------|
| Nuts with husks removed | Nuts with husks (husk, shell, kernel) | Nuts with husks removed |
| 10.80 | 11.42 | 7.61 | 3.59 | 46.06 | 42.48 | 30.65 | 39.57 | 10.80 | 11.42 | 7.61 |
| 37.62 | 39.57 | 46.06 | 42.48 | 30.65 | 39.57 | 10.80 | 11.42 | 7.61 | 3.59 | 3.59 |
| 12.19 | 10.80 | 11.42 | 7.61 | 3.59 | 10.80 | 11.42 | 7.61 | 3.59 | 3.59 | 3.59 |
| 5.10 | 3.16 | 12.42 | 4.39 | 1.76 | 5.10 | 3.16 | 12.42 | 4.39 | 1.76 | 1.76 |

the ash content (HAAS and BATCHELOR 1). During July and August the husks greatly increase in ash content, due in part no doubt to increased transpiration. The percentages of sodium and potassium in the ash of the husks increase with their age, while those of calcium and magnesium decrease. The amount of sodium and potassium, and that of calcium and magnesium, in the dry matter of the walnuts with husks removed, decreases with advancing age of the nuts, owing to the decrease in the percentage of ash. Sodium and potassium greatly increase, and the calcium decreases in the ash of walnut husks at a time when the leaves are increasing the percentage of calcium in their ash. The percentage of potash in the ash of walnut leaves decreases, while that of calcium increases with increasing age of the leaves. This is just the opposite of what happens in walnut husks.

In view of the extremely high amount of bases in the walnut husk, the following questions may be asked, although at present we cannot answer them: (1) Does the large amount of bases continue to increase until the death and splitting open of the husk follows as a consequence? (2) Is the walnut husk a dumping ground or an outlet for surplus absorption of bases? (3) Does the production of walnut fruits depend on such a surplus of bases in the husks? These questions indicate but a few of the many problems that have arisen.

EFFECT OF SALINE IRRIGATION WATER ON WATER SOLUBILITY OF INORGANIC CONSTITUENTS OF DRY MATTER OF WALNUT HUSKS

It is of interest, in connection with the large concentration of bases in walnut husks, to study the water solubility of the husks of young and of nearly mature walnuts, and to observe the changes that take place in this solubility when husks are taken from trees injured as a result of salinity of the soil. Table XVIII gives data which show that the ash of the husks from trees in saline soil may be less than that from trees in good soil. The ash of the water-soluble fraction as a percentage of the total ash increases with the age of the husks. The total calcium, as a percentage of the total ash, also decreases with increasing age of the husks. There is a larger percentage of the total calcium in the ash of the water-soluble fraction when

TABLE XVIII
EFFECT OF ABSORBED SALTS ON WATER-SOLUBILITY OF INORGANIC CONSTITUENTS OF DRY MATTER OF WALNUT HUSKS

| | HUSKS OF YOUNG WALNUTS FROM SALT-INJURED TREES, IRVINE, JUNE 3, 1925 | | HUSKS OF YOUNG WALNUTS FROM GOOD TREES, IRVINE, JUNE 22, 1925 | | HUSKS OF WALNUTS FROM TREES 16-18 IN. IN SOIL RECEIVING SALINE IRRIGATION WATER, SHEET 91 1925 | | HUSKS OF WALNUTS FROM GOOD TREES, IRVINE, AUGUST 15, 1925 | |
|--|--|----------------------|---|----------------------|--|----------------------|---|----------------------|
| | Soluble fraction % | Insoluble fraction % | Soluble fraction % | Insoluble fraction % | Soluble fraction % | Insoluble fraction % | Soluble fraction % | Insoluble fraction % |
| Ash of different fractions as percentage of dry matter | 3.49 | 1.34 | 5.51 | 1.58 | 8.77 | 0.84 | 10.46 | 2.26 |
| Ash of soluble fraction as percentage of ash | 72.31 | 77.68 | 11.49 | 7.95 | 91.26 | 2.31 | 82.22 | 5.37 |
| Total Ca as percentage of total ash | 18.41 | 81.59 | 4.96 | 95.04 | 19.37 | 80.63 | 4.10 | 95.90 |
| Percentage of total Ca in each fraction | 2.93 | 33.86 | 0.51 | 33.84 | 0.49 | 25.31 | 0.27 | 28.97 |
| Ca. of each fraction as a percentage of ash of same fraction | 78.06 | 21.94 | 75.90 | 24.10 | 76.07 | 23.93 | 70.83 | 29.17 |
| Percentage of total Mg in each fraction | 94.20 | 5.80 | 4.06 | 95.94 | 2.75 | 2.43 | 1.88 | 1.88 |
| Total Mg as percentage of total ash | 97.01 | 2.99 | 81.70 | 18.30 | 68.21 | 31.79 | 56.49 | 43.51 |
| Percentage of total Na. in each fraction | 97.01 | 2.99 | 1.43 | 2.15 | 2.03 | 1.03 | 1.03 | 1.03 |
| Total Na as percentage of total ash | 97.01 | 2.99 | 96.75 | 3.25 | 97.50 | 2.50 | 95.31 | 4.69 |
| Percentage of total K in each fraction | 29.14 | 35.53 | 35.53 | 45.31 | 45.31 | 43.40 | 43.40 | 43.40 |
| Total K as percentage of total ash | 29.14 | 35.53 | 35.53 | 45.31 | 45.31 | 43.40 | 43.40 | 43.40 |

the husks are from salt-injured trees than when they are from good trees, regardless of age as a factor. The soluble calcium as percentage of the ash of the soluble fraction is greatest in each case when the husks are from salt-injured trees. There is more total magnesium as percentage of the total ash and a greater percentage of the total magnesium, sodium, and potassium in the water-soluble form in the husks from salt-injured trees, than in those from good trees. The concentration of potassium increases with age, and over 95 per cent of it is water-soluble.

JUICE OF WALNUT HUSKS AND YOUNG KERNELS

Analysis of the ash of the juice of walnut husks (table XIX) shows a large concentration of some of the soluble salts, and indicates a low concentration of divalent bases in comparison with the

TABLE XIX

COMPOSITION OF JUICE OF WALNUT HUSKS AND THAT OF YOUNG WALNUT KERNELS

| MATERIAL | CONDITION OF TREES | COLLECTED | SAMPLE (cc.) | PH | PARTS PER MILLION | | | | | | | |
|-------------------------------|--------------------|---------------|--------------|-----|-------------------|-------|------|-----|-----|-----|-----------------|-----------------|
| | | | | | Ash | Na | K | Ca | Mg | Cl | SO ₄ | PO ₄ |
| Juice of walnut husks | Normal . . . | June 23, 1926 | 100 | ... | 6881 | 868 | 2713 | 76 | 306 | 537 | 1254 | ... |
| Juice of young walnut kernels | Salt affected | May 25, 1925 | 14 | 5.3 | 500 | 4184 | 440 | ... | ... | ... | ... | ... |
| | Salt affected | May 25, 1925 | 12 | 5.4 | 702 | 3741 | 383 | ... | ... | ... | ... | ... |
| | Salt affected | June 22, 1925 | 28 | 5.2 | 10900 | 10944 | 4820 | 350 | 207 | 43 | 162 | 1173 |
| | Salt affected | June 22, 1925 | 10 | 5.2 | 10700 | 2490 | 4494 | 460 | 320 | 60 | 74 | 1490 |
| | Normal . . . | June 22, 1925 | 45 | ... | 1167 | 3758 | 269 | 177 | ... | ... | ... | ... |

monovalent ones. A high concentration of phosphate is also evident. By careful manipulation, it was possible to remove kernels from walnuts while still in the liquid condition, and then secure the liquid by puncturing the kernel. The 10 cc. and 28 cc. samples were ashed and the ash was analyzed; whereas in the other samples direct aliquots of the juice were made for analysis of the various constituents. It was not possible to determine chlorine directly in the fresh juice, because of the reducing action of the juice on silver nitrate.

The juice of kernels obtained at Irvine, California, always gave pH values close to 5.2, which is the same as that found by HOAGLAND and DAVIS (4) for the sap of *Nitella* cells. In *Nitella* sap, as compared

with walnut kernel liquid, the monovalent bases are lower, the divalent bases about the same, the phosphate lower, and the chlorine and sulphur higher. The specific conductivity of the juice obtained from walnut kernels collected June 21 correspond at 25° C. approximately to that of an N/10 solution of potassium chloride. Titration of the juice with N/10 NaOH, with phenolphthalein as indicator, showed that its acidity corresponded approximately to an N/40 acid. Table XIX shows the large concentrations of the constituents of the clear, colorless juice of the young kernels. This juice from salt-affected trees contained larger concentrations of sodium, potassium, calcium,

TABLE XX

TOTAL S AND P OF WALNUT KERNEL JUICE AND EXPRESSED JUICE
OF WALNUT HUSKS (JUNE 22, 1926)

| | S (GM.) | P (GM.) |
|--|---------|---------|
| 15 cc. walnut kernel liquid with $Mg(NO_3)_2$ added..... | 0.0023 | 0.0026 |
| 15 cc. walnut kernel liquid without $Mg(NO_3)_2$ added..... | 0.0009 | 0.0025 |
| 10 cc. juice (extracted by crushing husks of good walnuts) with $Mg(NO_3)_2$ added..... | 0.0016 | 0.0019 |
| 10 cc. juice as preceding but no $Mg(NO_3)_2$ added..... | 0.0016 | 0.0015 |

and magnesium than that from normal trees. The sodium of the kernels from the salt-affected trees showed a marked increase with increasing age of the kernel. The changes which the constituents of the kernels undergo in their later development deserve study.

The volatility of sulphur and phosphorus upon ashing the juice of husks and of young kernels still in the liquid condition is shown in table XX. In the kernel juice the sulphur was largely volatile upon ignition, whereas in the husk juice none was lost in this way. Very little of the phosphorus was lost during the ashing process.

VOLATILITY OF SULPHUR AND PHOSPHORUS OF WALNUT KERNELS ON ASHING

The great loss of sulphur that occurs when mature kernels are ashed is seen in table XXI. The loss in phosphorus upon ignition is also large, but the percentage loss is much less than that of sulphur. Much of the sulphur and phosphorus of walnut kernels exists in organic combination and is easily volatilized upon ignition.

EFFECT OF ALKALI SALTS ON COMPOSITION OF WALNUT KERNELS

The effect of alkali salts on the composition of walnut kernels is seen in table XXII. The dry matter of kernels from nuts of trees in saline soil had a higher ash content than that from trees on good

TABLE XXI

TOTAL SULPHUR AND PHOSPHORUS CONTENT OF MATURE WALNUT KERNELS

| LOCALITY | CONDITION OF TREES | GRAMS IN 100 GM. OF DRY MATTER | | | |
|------------------------|--------------------------|-----------------------------------|---------------------------------------|-----------------------------------|---------------------------------------|
| | | SO ₄ from ash analyses | SO ₄ from total S analyses | PO ₄ from ash analyses | PO ₄ from total P analyses |
| Irvine..... | Good | 0.018 | 0.680 | 1.110 | 2.273 |
| Trees 13-15, Riverside | Good | 0.017 | 0.628 | 1.095 | 1.865 |
| Irvine..... | Good | 0.011 | 0.640 | 1.105 | 1.718 |
| Irvine..... | Badly affected by alkali | 0.024 | 0.722 | 1.085 | 2.035 |

TABLE XXII

EFFECT OF ALKALI SALTS ON COMPOSITION OF WALNUT KERNELS

| | KERNELS FROM GOOD WALNUTS, IRVINE, SEPT. 18, 1925 | KERNELS FROM TREES ON ALKALI SOIL, IRVINE, SEPT. 18, 1925 | KERNELS FROM HUSKS WITH BURNED AREAS AND CHLORIDE ON SURFACE, FROM TREES ON ALKALI SOIL, IRVINE, SEPT. 18, 1925 |
|--------------------------------------|---|---|---|
| Ash as percentage of dry matter..... | (2.07) | (2.75) | (3.39) |
| Ash constituents (percentage of ash) | | | |
| Na..... | 4.60 | 4.04 | 3.98 |
| K..... | 25.41 | 26.57 | 30.44 |
| Ca..... | 4.19 | 3.74 | 5.10 |
| Mg..... | 5.20 | 5.96 | 9.47 |
| Cl..... | 0.51 | 2.08 | 3.94 |
| SO ₄ | 0.49 | 0.86 | 2.47 |
| PO ₄ | 53.29 | 39.36 | 49.18 |
| Fresh weight (gm.)..... | 86.0 | 130.0 | 78.0 |
| Dry weight (gm.)..... | 64.0 | 90.0 | 35.0 |

soil, owing to the fact that kernels from trees on saline soil have a lower content of dry matter than those of trees on good soil. The percentages of each of the bases in the dry matter, as calculated from the ash analyses of table XXI, are higher in the kernels from the trees on saline soil. The percentage of chlorine and sulphate in the ash is greatest for the trees on saline soil.

FURTHER INVESTIGATION

Very little is known about the seasonal or the cyclic changes in the inorganic relationship in fruit trees, although much is known regarding the migration of constituents of leaves during senility and just prior to abscission. The nature of the balance between the inorganic constituents of the leaves and of the fruit has received very little attention heretofore, investigations having concerned themselves largely with the carbohydrate-nitrogen ratio. While there is need of further study on the inorganic physiology of healthy walnut trees, the present studies have made some effort in this direction, and have also compared such data in many cases with the situation that arises when there are unusual salt effects of the environment upon the inorganic constituents, growth, and behavior of the trees.

Summary

1. The effects of saline irrigation water upon walnut trees have been studied with reference to the effects upon behavior and growth, and upon the relationship between the inorganic constituents.
2. The continued application of saline irrigation water, to tanks of soil planted to walnut trees, has resulted in a reduced size of the leaves with occasional leaf burn, but no indications of mottle leaf or of walnut "yellows."
3. There exists a gradient in the ash content of the dry matter of walnut trees, which in good soil has its minimum in the rootlets but in saline soil has a minimum near the level of the surface of the soil.
4. The production of a new cycle of growth brings about changes in the relationships of the inorganic constituents of the water-soluble and insoluble fractions of the dry matter.
5. Although the sodium, potassium, and calcium contents of the drainage water of artificially made saline soil, in which walnut trees were grown, were much greater than the magnesium content, yet the woody portion and leaves of the trees contained less sodium, potassium, and calcium but more magnesium than the corresponding parts of control trees in good soil.
6. Walnut trees may absorb large amounts of chlorine and sul-

phur, much of which may be in organic combination. Persimmon leaves, and citrus leaves and fruit, as well as walnut leaves and fruit have been shown to be able to absorb large amounts of chlorine and sulphur, and to place much of it in organic combinations. The dry matter of leaves of walnut trees growing in soils rich in chlorine may lose one-half or more of its chlorine upon gentle ignition, whereas practically none is lost in the case of badly burned mature walnut husks.

7. Saline soils affect not only the inorganic constituents of the trunk, shoots, and leaves, but also those of the husk and kernel.

8. The effects of the artificially made saline soil upon walnut trees and their fruit are not confined alone to accumulations of chlorine or sulphur, but involve equally important changes in the relationships between the bases within the tissues.

9. When a soil was artificially made saline in controlled tank cultures of walnut trees, the effect of salinity of the soil solution was in the direction of reducing rather than increasing the ash content in the portions of the plant above the soil level, as compared with homologous portions of suitable control trees.

10. The effect of direct sunlight upon walnuts is to bring about an increase in the ash constituents of the dry matter. Such effects, when extreme temperatures also occur, may be so destructive in their action as to cause burning of the husks with its consequent effects upon the marketability of the nuts.

11. Absorption from salt solutions by short shoots with walnuts attached, while the kernels were still in the liquid condition, resulted in a considerable increase in sodium in the kernel portion. Although a considerable amount of chlorine and sulphur was absorbed by the shoots into the walnuts, only the increased sulphur of the husks was retained upon ignition.

12. The ash content of walnut husks increases most rapidly from July to late summer. Mature husks are extremely rich in bases, but the importance of such high concentrations in the physiology of the husk is very little understood. The water solubility of the inorganic constituents of husks changes considerably according to the nature of the soil solution bathing the tree roots.

13. The pH of the juice of young walnut kernels still in the liquid condition was about 5.2. Such juice was extremely rich in inorganic constituents.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

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CYTOTOLOGY AND LIFE HISTORY OF VAUCHERIA GEMINATA

J. R. MUNDIE

(WITH PLATES XIII, XIV)

Introduction

It is generally agreed that the oogonium of *Vaucheria* is multinucleate when young, and in its mature stage uninucleate; but there is a difference of opinion concerning what happens to the numerous nuclei that occur in the young oogonium as it develops from the multinucleate into the uninucleate or mature condition.

Conflicting accounts of oogenesis in *Vaucheria* have been given on the one hand by OLMANNS (6) and on the other by DAVIS (2). OLMANNS found that all the nuclei except one withdraw from the oogonium of *V. sessilis* before the formation of the membrane which separates the oogonium from the parent filament. DAVIS contended that the nuclei remain in the oogonium of *V. geminata* until after the formation of the membrane, and that they begin to disintegrate just before the formation of the cross wall and continue to disintegrate very rapidly after its formation, until, by the time the cross wall is completely developed, the nuclei are hardly recognizable. OLMANNS' results were confirmed by HEIDINGER (4). In this respect DAVIS pointed out a resemblance in the behavior of the nuclei in gametogenesis in *Vaucheria* and in *Saprolegnia*, several members of the Peronosporales, and certain of the Ascomycetes.

DAVIS (2) also stated that the nuclei are carried into the young oogonium by the inflow of protoplasm. He found that their number varies from twenty to fifty. Furthermore, he stated that it is probable that mitotic figures are present in the vegetative branch and also in the earlier stages of the young oogonium, but he was unable to see the spindles. He concluded that there are no mitoses during the growth of the oogonium and none after separation by the cross wall. DAVIS suggested this as one of the points in which this process differs from that found in *Saprolegnia* and the Perono-

sporales, where there seem to be always one or two general mitoses after the oogonium is formed.

KURSSANOW (5) showed that mitosis, or nuclear division, occurs in the filament of *Vaucheria*, and that the number of chromosomes is rather small.

Material and methods

The first collection of the material from which most of the cytological results were obtained was made in October, 1924, from the banks of the Rivanna River near the Woolen Mills, Charlottesville, Virginia. For comparison, collections were made in the neighborhood of the University of Virginia, and from greenhouses. The first collection showed a high degree of reproductive activity, with numerous oogonia and antheridia in practically every stage of development. The species studied was *V. geminata*.

Some of the material was fixed in the field immediately upon collection. A second lot was brought into the laboratory, kept moist with as little disturbance as possible until 2:30 A.M. the following day, and then fixed. There were about 12 hours between the fixation of the first lot and that of the second. The fixing fluid consisted of sixteen parts of 1 per cent chromic acid and one part of glacial acetic acid. This was mixed with formalin, two parts of the first mixture to one of the formalin. To this fluid about 1 cc. of 1 per cent osmic acid was added to every 100 cc. of chromo-aceto-formalin mixture. The formalin and osmic acid were mixed with the chromic and acetic acids just before using.

The material remained in the fixing fluid for about 30 minutes. It was washed 4 hours in running water, then carried to 100 per cent alcohol by very slow changes in the weak alcohols, beginning with 2.5, 5, and 8 per cent, etc. The material remained at least 2 hours in each strength of alcohol, and was then imbedded in paraffin by gradual transfer through xylol and paraffin solutions, then sectioned. Sections were cut 5 μ and 7 μ thick.

Of a number of stains experimented with, safranin and gentian violet in 1 per cent solution gave the best results. Both resting nuclei and mitotic stages in the oogonium were excellently stained. The safranin in 50 per cent alcohol was first applied to the slide for 2 or 3 minutes, then gentian violet in 25 per cent alcohol was applied

for 2 or 3 minutes, and the slide quickly rinsed in 95 and 100 per cent alcohols. Clove oil was used for differentiation while the specimen was observed under a 4 mm. objective. This stain gave very satisfactory results in all stages of development of the oogonium.

Safranin in 50 per cent alcohol and light green in clove oil gave best results when the stains were used in terms of minutes and seconds rather than hours; however, this combination was not satisfactory except in occasional instances. Haidenhain's haematoxylin was not satisfactory because the chromatophores took the stain so deeply that the nuclei could not be differentiated and distinguished from them. Acid fuchsin and methyl green were used according to the schedule for safranin and gentian violet. This combination gave results almost as good as the latter. Delafield's haematoxylin used for 2-3 minutes was decolorized with acid alcohol and followed by alkaline alcohol. This stain was used only on unsectioned material, and gave excellent results.

Field observations

About the middle of March, 1926, *Vaucheria geminata* made its first flourishing appearance after the winter season. The period of vigorous growth lasted until about the first of May. Sexual organs developed during the latter part of April and the early part of May.

About the same time that *Vaucheria* was observed in its natural habitats, oospores were successfully germinated in the laboratory from cultures that had been kept there from the previous autumn. Fig. 16 shows a camera lucida sketch of a germinating oospore in the living condition. Other germinating oospores were observed, but not in sufficient numbers to section, and for the most part were older than the stage shown in the figure. Only one stage was observed younger than the one shown.

From June until August scarcely more than an occasional fragment of a filament could be found. This might have been due in part to the excessive dryness of the soil prevailing during this period. In the earlier part of July a 3-day period of rain and fog occurred, however, and following this period no signs of *V. geminata* could be found. During the first week in August another period of rain and fog occurred, similar to that of July. Within a week filaments of

Vaucheria could be observed with a hand lens. The plant increased in abundance until it almost covered the ground by the latter part of September. Sexual organs began to appear about the middle of this month, and were found in abundance during the earlier part of October. The sexual organs seemed to be more numerous in the fall than in the spring. The plant was green and appeared to be in good condition until injured by frosts the latter part of October. After heavy freezing weather, until about the middle of March, only fragments of living filaments could be found. These were always in close contact with the soil, indicating that *V. geminata* winters over, in part at least, by means of these fragments which resist freezing.

Field observations in 1925 gave the same results. *V. geminata* has two periods of vigorous development, one in the spring, from March to May, the other in the late summer and early fall, from August to the time of heavy frost. In the second period of activity the plant is more vigorously developed than in the first.

Cytological observations

The oogonia and antheridia arise from a process which branches off the main filament. Both male and female organs are borne on the same stalk. The oogonia arise as swellings of the tips of lateral filaments, while the antheridium occupies a central position and continues as an elongation of the terminal portion of the original stalk, but somewhat more slender, curving downward between the oogonia. The antheridial cell is soon cut off from the remainder of the plant by a partition, the male elements developing in the cell thus formed. The oogonia increase rapidly in size, and at the same time show the presence of numerous vegetative nuclei. When the oogonium has attained considerable size, a mass of dense cytoplasm occurs in the apical portion, in which is located a single nucleus. This nucleus is much larger than the vegetative nuclei, and always occupies a position in the apical portion of the oogonium (fig. 2). The oogonium continues to increase in size, while the apical portion tends to curve downward. As the egg nucleus is being carried forward in the cytoplasmic mass large vacuoles arise between the center and the apex of the oogonium. The vegetative nuclei are left behind

the vacuolated area. They show no signs of migrating either to or from the oogonium.

One or two periods of general mitosis occur in the oogonium when nearly all the nuclei are in some phase of division. The egg nucleus, which has been set off from the vegetative nuclei in a dense mass of cytoplasm, shows mitotic stages in advance of those in the vegetative nuclei; that is, when the gamete nucleus is in anaphase the vegetative nuclei are in metaphase or earlier stages of mitosis. The gamete nucleus undergoes a mitosis which strongly indicates a reduction phenomenon. Figs. 7 and 7a show a gamete nucleus in the anaphase. Five distinct chromosomes are in one group while there are five in the other group less distinctly visible. Distinct metaphases were observed in the vegetative nuclei of the same oogonium (figs. 6, 6a, 6b). Ten chromosomes were observed in the metaphase of a vegetative nucleus (figs. 6, 6a); hence the grouping of the chromosomes into fives in the gamete nucleus probably indicates a reduction condition. Another vegetative nucleus in the same oogonium shows the chromosomes on the equatorial plate with distinct spindles and no nuclear membrane present. A centrosome is plainly visible at one of the poles, while it is very faint at the other (figs. 6, 6b).

Nuclei destined to become female gamete nuclei are shown in figs. 2, 2a, and 3. These nuclei, from young oogonia in which there is no trace of a cross wall, are certainly in an early prophase condition, as indicated by the large granules of chromatin scattered throughout the nucleus and by the absence of a karyosome. The vegetative nuclei, so far as could be determined, are all in a resting condition in the oogonia from which these drawings were made.

Figs. 3, 2a, 4a, and 7a represent a series of stages in the development of the egg nucleus. In fig. 3 an early prophase with about twenty distinct chromatin granules may be seen. At a later stage these granules are seen (fig. 2a) to be aggregated into less than ten larger masses. This is a stage in the organization of bivalent chromosomes, which is better seen in the anaphase shown in figs. 7 and 7a. After mitosis is complete it is seen that five chromatic bodies remain in the mature egg nucleus, while the remainder have passed into a small mass applied like a cap to the side of the egg nucleus

(fig. 4a). The whole process bears a strong resemblance to polar body formation.

The method of formation of the cross wall conforms to the description of DAVIS (2), except that it does not take place until after fertilization. The origin of the cross wall is also very similar to the same phenomenon described by HARPER in *Pilobolus* and *Sporodinia*; by SWINGLE (9) in *Rhizopus* and *Phycomyces*; and by YAMANOUCHI (12) in *Polysiphonia*. After the male element enters the oogonium, vacuoles begin to form in the region where the cross wall is to appear (fig. 13). These fuse until they form a partition across the stalk of the oogonium, and finally separate it from the parent filament. The cross wall is formed along the boundaries of the large flat vacuole, which now completely separates the oogonium and the filament.

There is no evidence in the writer's preparations in support of OLTMANNS' contention that there is a migration of nuclei from the oogonium before the formation of the cross wall. At the time of fertilization the vegetative nuclei are as abundant in the oogonium as at any other time. The membrane of the partition begins to form immediately after fertilization, and in all probability is completely formed by the time fusion of the male and female nuclei occurs. Evidence is in support of DAVIS' contention, that the nuclei begin to disintegrate about the time of the beginning of the formation of the cross partition. Before fusion of the gamete nuclei (fig. 14), resting nuclei are still present, although many seem to have lost their nuclear membrane in a manner comparable with the description of DAVIS. Fig. 15 shows a condition in which the gamete nuclei have fused, the fusion nucleus has migrated to the center of the oospore, and of the vegetative nuclei, only a trace of chromatin material here and there is visible. The cross wall has completely formed in this condition.

When fertilization occurs, the large vacuoles (which have been occupying a more or less centro-apical position) become smaller, and a structure (figs. 13, 14) which seems to have cytoplasmic connections with the dense cytoplasm about the gamete nuclei is formed. When the male nucleus has attained a size nearly equal to that of the female, the gamete nuclei fuse and the vacuolated structure

and dense cytoplasm disappear. Finally the zygote nucleus occupies a position in the center of the oospore.

A coenocentrum is not present in *V. geminata* unless the dense mass of cytoplasm in which the egg nucleus is located be homologized with such a structure. This is a mass of granular cytoplasm which differs from the rest of the cytoplasm by being more dense and not vacuolar. It is not a spherical structure, but has a more or less definite semiellipsoidal shape. In this the egg nucleus undergoes mitosis. This structure does not occur in the center of the oogonium, as has been described by WAGER (11) and others as characteristic of coenocytic plants. It is located near the apical portion of the oogonium just behind the "receptive spot." It is certainly a dynamic center of activity, as is shown by the behavior of the egg nucleus.

The writer's preparations show on the average a considerably greater number of vegetative nuclei in an oogonium than DAVIS (2) estimated. He stated that the range was from about 20 to 50; I find from several counts that the range in my material is from 55 to 141. It is probable that DAVIS' counts were made in young oogonia, before the number of vegetative nuclei was increased by mitotic division. DAVIS was of the opinion that the nuclei are carried into the young oogonium by the inflow of the cytoplasm; evidence from my material indicates that there is no nuclear migration to or from the oogonium in *V. geminata*.

Earlier investigators were of the opinion that all of the vegetative nuclei in an oogonium are potentially gamete nuclei. I am convinced that this does not hold true in the case of *V. geminata*. The gamete nucleus is set off from the vegetative nuclei early in the development of the oogonium. It occupies an apical position with respect to the oogonium, and is always seated in a dense granular mass of cytoplasm in which no vegetative nuclei are found. At the same time the vegetative nuclei occupy the two-thirds of the oogonium nearest the stalk. This indicates that the gamete nucleus is distinctly differentiated from the vegetative nuclei early in the development of the oogonium.

The problems of the time of differentiation of the gamete nucleus and of its relation to the vegetative nuclei are of considerable importance and difficulty. The oogonia arise as swellings of the tips

of lateral filaments borne on a stalk branching from the main filament, and are in a sense comparable with the tips of vegetative filaments. In actively growing points the vegetative nuclei are more abundant near the apical portion than farther back in the filament (fig. 17). At the same time many specimens show that the cytoplasm is more dense near the tip, and resembles in appearance the cytoplasm found in the "receptive region" of the oogonium. In this mass several nuclei are located.

The phenomenon of mitosis makes itself apparent in the oogonium of *V. geminata* when it has nearly reached its full size. The egg nucleus lies in a dense mass of cytoplasm just behind the receptive region. It is considerably larger than the vegetative nuclei, and no vegetative nuclei are found near it. Before mitosis is distinguishable in the vegetative nuclei, they all present a typical resting condition in having a large karyosome, more or less centrally located, and a clearly outlined nuclear membrane. The enlarged egg nucleus has the chromatin scattered throughout it in the form of numerous small granules (fig. 3). This condition is followed by a decrease in the number of granules present in these stages. A metaphase of the egg nucleus has not been observed. Fig. 7 shows a section of an oogonium containing the egg nucleus in a typical anaphase. Five chromosomes are in one group, while the other five are in a denser group and are less distinctly stained. Another section from this same oogonium (fig. 6) shows the vegetative nuclei in prophase and metaphase stages, and a few resting nuclei. Fig. 6a shows a vegetative nucleus in which ten chromosomes were counted. The enlarged drawing shows these chromosomes and their relation to each other as sketched with a camera lucida under considerably higher magnification. No spindle fibers were visible in this mitotic figure. Fig. 6b, however, under higher magnification, shows a vegetative nucleus with distinct spindle fibers and two centrosomes, one less distinct than the other. This situation shows that the egg nucleus undergoes mitosis, and that it is in a more advanced stage than any of the vegetative nuclei in the same oogonium. A still more advanced stage of mitosis in the egg nucleus, a late telophase, is shown in figs. 4 and 4a. The egg nucleus has its membrane, but on one side is a distinct swelling containing a mass of chromatin. The egg nucleus

proper shows several large chromatin granules scattered through it. The vegetative nuclei are in late anaphases and early telophases.

While these nuclear changes are taking place, the central portion of the oogonium is becoming filled with large vacuoles. Fig. 5 illustrates another section of the same oogonium as fig. 4, showing the vegetative nuclei in late stages of mitosis and a few in the resting condition. The vegetative nuclei are seen to be much more numerous here than in fig. 2. Whether there have been one or two periods of general mitosis between the stages represented, in these figures is impossible to say; however, eighteen vegetative nuclei are present in the section of fig. 2, while there are about forty-five in fig. 5. When the mitosis is completed, in the section shown in fig. 5, there will be something like seventy-five or eighty vegetative nuclei present. This situation shows that if there were approximately the same number of vegetative nuclei present in the oogonium of fig. 5 as were present in the oogonium of fig. 2 when the two oogonia were in the same stage of development, then certainly two periods of general mitosis must have occurred in the oogonium of fig. 5 in order to account for the increase as indicated. The vacuolated structure of the oogonium and the presence of a large number of vegetative nuclei, many of which are in stages of mitosis, indicate that the development of the egg is complete or nearly so. Figs. 4, 4a, 5, 7, and 7a support this view.

The process of fertilization seems to be effected by the antheridium and oogonium coming in close contact. A swelling occurs upon the apical portion of the oogonium, probably due in part to the wall of the oogonium becoming thin in that region (figs. 8-10). This condition was observed in living as well as in fixed material. The structure resembles somewhat that described by STEVENS (8) for *Albugo bliti*, and (7) for *Albugo portulacae*, and termed the "receptive papilla."

The phenomenon of fertilization was observed only in material fixed at 2:30 A.M., while mitotic figures were found only in the egg nuclei of material fixed at 2:30 P.M. This would indicate that during the afternoon the vegetative nuclei and the egg nucleus undergo mitotic divisions; while fertilization, the formation of the cross wall, and the maturing of the zygote occur in the latter part of the night.

and early morning. When the male nucleus enters the oogonium it advances toward the egg nucleus (fig. 11), but does not fuse with it immediately (figs. 14, 8, 8a); it lies close beside it, and there grows until it has reached approximately the size of the female gamete nucleus (fig. 14). Then fusion occurs, while the fusion nucleus passes toward the center of the oosphere (fig. 15).

Discussion

Vaucheria geminata shows periodic occurrence. Whether the controlling factor is length of day or temperature is a point of interest. The seasons of spring and autumn are without doubt most favorable for its development, both vegetatively and reproductively. Observations have been made on material growing under natural conditions. The same general conditions seem to exist in every case. *V. sessilis* was observed to be in a high state of vegetative development in a greenhouse in midwinter, while *V. geminata* a few feet away was barely living and presenting a yellowish green appearance, with only enough of the filaments present to make it apparent to the naked eye. Why was *V. geminata* not in a vigorous stage of development while *V. sessilis* was, under the same conditions? Similar observations were made during the summer months from June to August, and a condition was found comparable with that of the midwinter season.

Observations on photoperiodism by GARNER and ALLARD (3) indicate the possibility that *V. geminata* in its periodical development may be influenced by the length of the day. Comparing the time between sunrise and sunset of the two periods when reproduction is becoming fairly evident, we find that the length of the day in spring (April 15) is 13 hours, 13 minutes; in the fall (September 15) it is 12 hours, 28 minutes. Hence these observations indicate that a day of approximately 12.75 hours is the optimum one for vigorous development of *V. geminata*.

Anyone undertaking a study of this plant will encounter serious difficulty in obtaining material in the proper vegetative and reproductive stages to secure evidence on every point presented in this paper. In this work the principal material from which these studies

were made was collected about the middle of October, and treated as previously described. Many other collections were made at various times, but in no case were they so favorable for study. Probably this condition accounts for the difficulty experienced by earlier investigators in correctly interpreting the actual conditions that accompany sexual reproduction in *Vaucheria*. Previous investigators were more or less interested in some one point and overlooked or misinterpreted many others. For example, DAVIS (2) was chiefly concerned with disproving OLTMANNS' theory of the migration of the nuclei from the oogonium, and to secure some points of similarity between *Vaucheria* and certain of the fungi. Since DAVIS worked with the same species that I have worked with, *V. geminata*, it is evident that the material with which he worked lacked some of the critical stages, or that he overlooked them if they were present. The position of the egg nucleus, the absence of mitotic figures, and other points which he described concerning the oogonium are quite different from those found by the writer. OLTMANNS' theory of the migration of the nuclei from the oogonium before the formation of the cross partition has no support from my observations, even though his theory has been confirmed by a student of his. Possibly the situation is different in *V. sessilis*, but from the few preparations made of this material I find no evidence supporting his contention.

DAVIS's theory of the vegetative nuclei flowing into the oogonium with the inward flow of the cytoplasm gets no support from my observations. Strong evidence is obtained from the previous study that the progenitors of the gamete nuclei are located in the apical portion of the growing filament. All of the nuclei, both vegetative and reproductive, found in the oogonium arise as a result of mitotic divisions occurring within the oogonium. Early in the development of the oogonium the nucleus destined to become the gamete nucleus becomes surrounded by a dense mass of cytoplasm. As the oogonium grows, the egg nucleus is carried forward in this mass, leaving the vegetative nuclei behind. The vegetative nuclei occupy the proximal two-thirds of the oogonium, while the egg nucleus lies in the distal third. When the egg nucleus has completed the last maturation division, the vegetative nuclei have attained their greatest number

within the oogonium. The fact that many vegetative nuclei arise after the functional egg nucleus has become differentiated overthrows an old idea that all of the vegetative nuclei are potential gamete nuclei.

Summary

1. *Vaucheria geminata* is found most abundantly in the spring and autumn. Sexual reproductive organs are most abundant in September and October; they are also found about April 15.
2. There are one or two periods of general mitosis in the oogonium. The egg nucleus undergoes mitosis just in advance of the vegetative nuclei.
3. The nuclear membrane disappears in both the vegetative and reproductive nuclei during mitosis. Spindle fibers are visible in the vegetative nuclei in the metaphase.
4. The cross wall begins to form about the time the sperm enters the oogonium, and is of vacuolar origin. The vegetative nuclei begin disintegration at the time of fertilization.
5. There is no migration of nuclei either to or from the oogonium of *Vaucheria geminata*.
6. A swelling forms from the oogonium in the "receptive region," which comes in contact with the antheridium at the time of fertilization. The male element enters through this process.
7. Fertilization occurs during the latter part of the night. Maturation divisions in the egg nucleus occur in the afternoon. The formation of the cross wall, the disintegration of the vegetative nuclei, and the fusion of the gamete nuclei occur in the latter part of the night and early morning.
8. The number of vegetative nuclei in the oogonium varies in specimens examined from 55 to 141. They are increased considerably in number during the periods of mitosis.
9. The fusion of the gamete nuclei does not occur immediately when the male element enters the oogonium. The male nucleus lies beside the egg nucleus and there enlarges to approximately the size of the female gamete nucleus.
10. In the center of the oogonium a vacuolar structure forms after fertilization. The zygote nucleus takes its position in the midst of this structure.

Professor I. F. Lewis deserves special mention in connection with this work, for it was through his advice and suggestions, together with his close observation of preparations presented to him, that this work was made possible.

UNIVERSITY OF VIRGINIA
CHARLOTTESVILLE, VA.

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EXPLANATION OF PLATES XIII, XIV

PLATE XIII

FIGS. 1, 2, 4, 5, 6, 7 $\times 725$; figs. 2a, 3, 4a, 6a, 6b, 7a $\times 1850$.

FIG. 1.—Section of young oogonium.

FIG. 2.—Oogonium showing large female nucleus; no signs of a cross wall.

FIG. 2a.—Female nucleus of fig. 2, showing structure under higher magnification.

FIG. 3.—Early prophase condition of female nucleus.

FIG. 4.—Vegetative and reproductive nuclei in states of mitosis.

FIG. 4a.—Female nucleus of fig. 4 highly magnified.

FIG. 5.—Section from same oogonium as fig. 4, showing vegetative nuclei and their relation to stalk.

FIG. 6.—Section of oogonium showing vegetative nuclei in stages of mitosis: *a*, ten chromosomes in metaphase stage; *b*, distinct spindle fibers.

FIGS. 6a, 6b.—Vegetative nuclei highly magnified.

FIG. 7.—Female nucleus showing two groups of five chromosomes each.

FIG. 7a.—Female nucleus of fig. 7 highly magnified.

PLATE XIV

FIGS. 8, 9 $\times 425$; fig. 10 $\times 475$; figs. 8a, 12, 15 $\times 1850$; figs. 11, 13, 14, 15 $\times 725$; fig. 16 $\times 50$.

FIGS. 8-10.—Sections from same oogonium; note position of antheridium, also vacuoles in center and in region of cross partition of oogonium.

FIG. 8a.—Gamete nucleus of fig. 8 highly magnified.

FIG. 11.—Receptive region of oogonium with egg nucleus and approaching sperm.

FIG. 12.—Nuclei of fig. 11 highly magnified.

FIG. 13.—Oogonium with male and female nuclei in close contact, showing numerous vacuoles in region where cross wall will form; note disintegrating condition of vegetative nuclei with the loss of membranes.

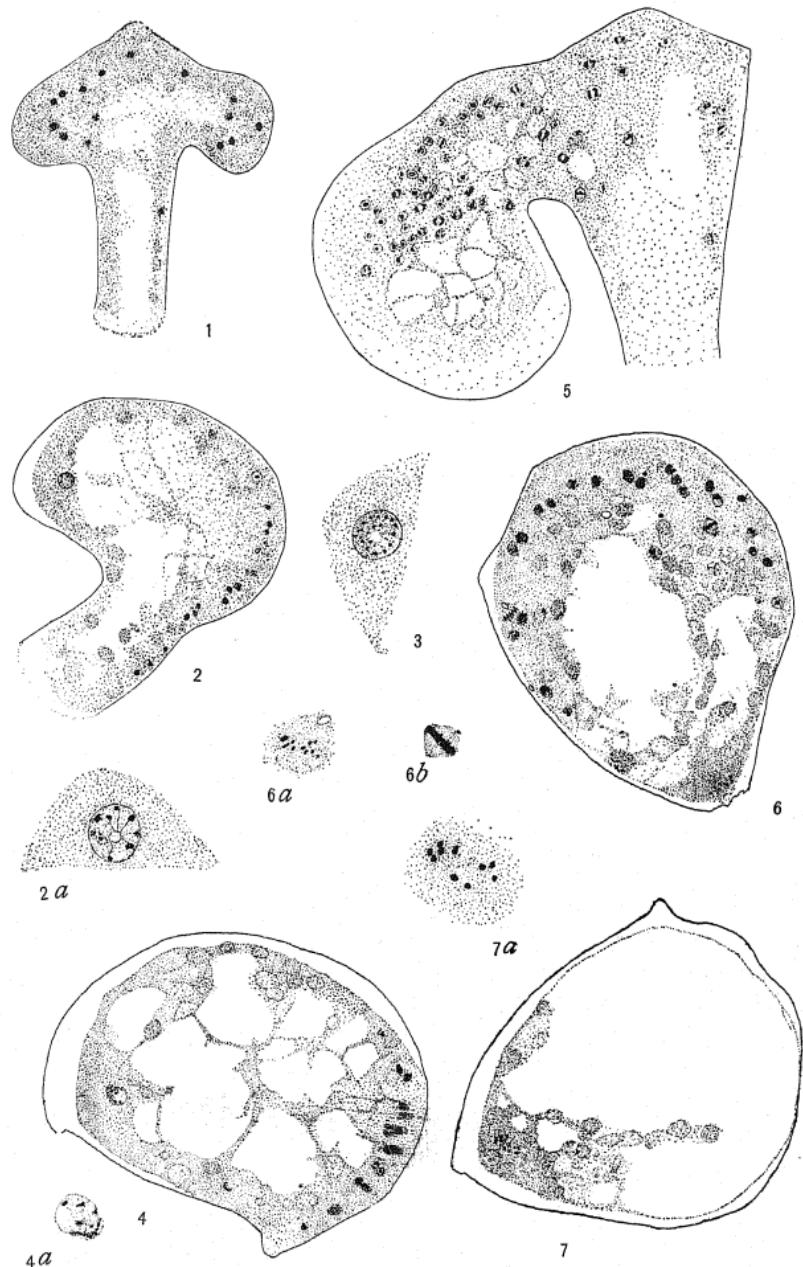
FIG. 14.—From an *in toto* preparation stained with Delafield's haematoxylin; male and female gamete nuclei approximately same size; vacuoles numerous in center of oogonium; vegetative nuclei in stages of disintegration.

FIG. 15.—Mature zygote at center of oosphere; cross wall completely formed; fragments of vegetative nuclei observable.

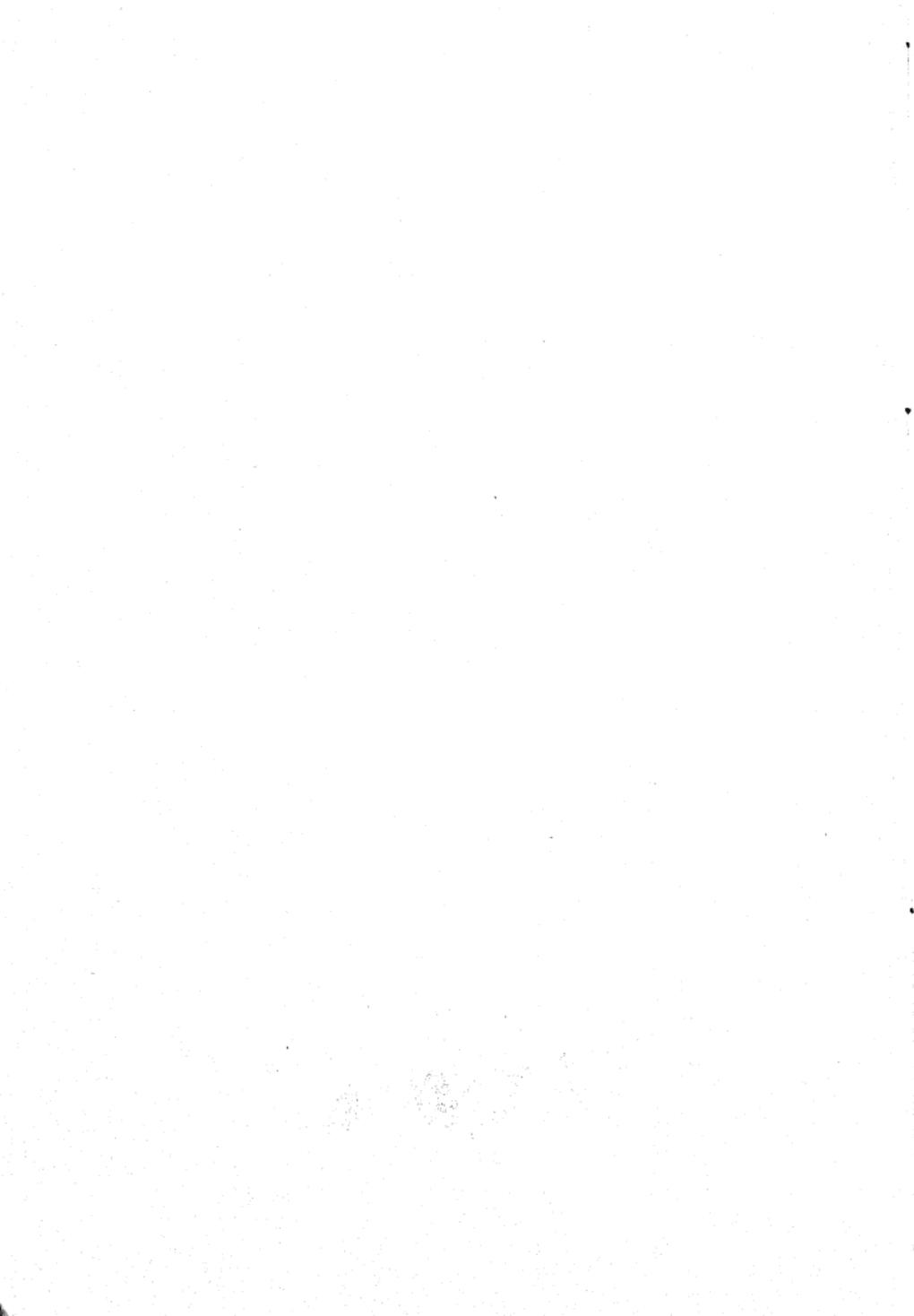
FIG. 15a.—Zygote of fig. 15 more highly magnified; note structure of nucleus.

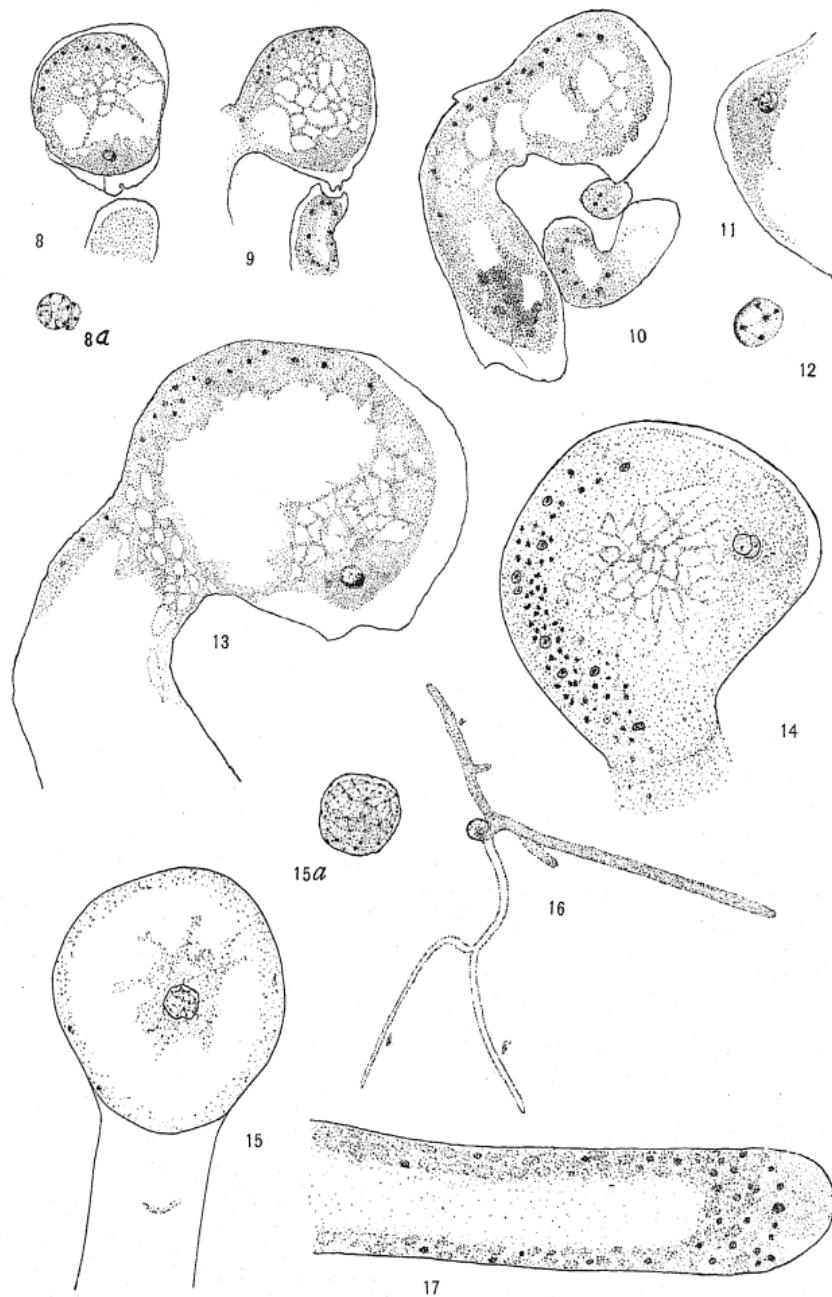
FIG. 16.—Germinating oogonium with old hull still clinging to base: *a*, *a'*, filaments; *b*, *b'*, rhizoids.

FIG. 17.—Tip of young vegetative filament; note numerous nuclei near growing tip.



MUNDIE on VAUCHERIA







DEVELOPMENTAL HISTORY OF THE FRUIT IN LINES OF CUCURBITA PEPO DIFFERING IN FRUIT SHAPE

EDMUND W. SINNOTT AND GEORGE B. DURHAM

(WITH ONE FIGURE)

Introduction

Investigations on the heredity of form of leaves, fruits, and other organs in various species of plants show in most instances that characters of this sort follow the same method of inheritance as do other quantitative and qualitative traits. The problem is somewhat complicated here, however, by the fact that what is inherited is not a simple character which may be measured directly, but consists in a ratio between two or more dimensions, and thus involves the general problem of growth correlations and their inheritance. Careful quantitative studies of heredity in such traits have not been numerous, but are greatly to be desired in view of the light which they may throw on developmental problems.

The senior author^{1, 2} has for some time been studying the inheritance of fruit shape in *Cucurbita pepo*, as measured by dimensional indices for the fruit as a whole and for its various parts, and has attempted to analyze his results in Mendelian terms. On the assumption of the operation of genetic factors which control various degrees of flattening or elongation, and which display the familiar phenomena of dominance, segregation, independent assortment, linkage, and cumulative and inhibitory effects, it has been found possible to explain the results of fruit shape inheritance in this species in a somewhat satisfactory manner. Studies of the same sort by LEAKE, KEARNEY, LINDSTROM, and others have led to similar conclusions for their material. Particular attention was paid to the earlier developmental stages.

¹ SINNOTT, E. W., Inheritance of fruit shape in *Cucurbita pepo*. *BOT. GAZ.* 74:95-103. 1922.

² ———, A factorial analysis of certain shape characters in squash fruits. *Amer. Nat.* 61:333-344. 1927.

A genetic analysis of such traits no longer satisfies students of heredity, however, who are increasingly concerned with the developmental phases of their problems, and are endeavoring to under-

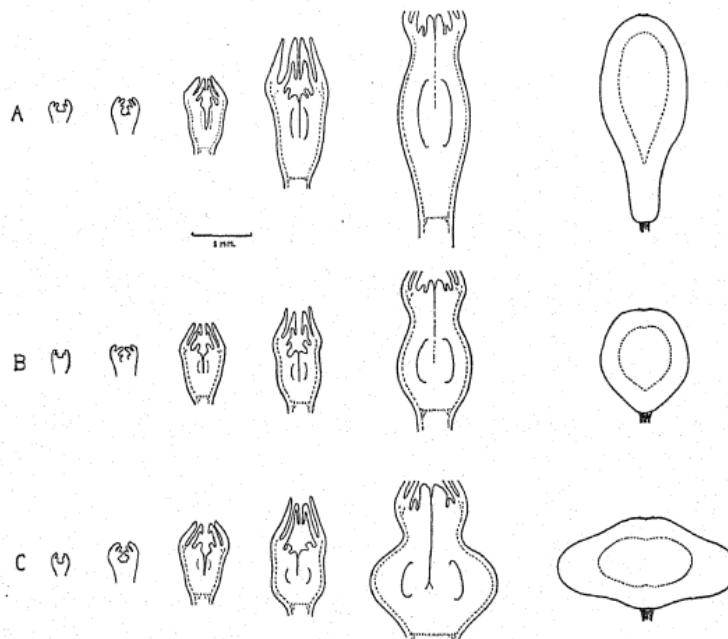


FIG. 1.—Outlines, from camera lucida drawings, of longitudinal sections through successive stages in development of ovary and fruit in elongate type (A), spherical type (B), and disk type (C) of squash fruits. First five stages drawn to scale. First shows early floral primordium and rudiments of sepals and petals; second shows beginning of carpillary cavity and rudiments of staminodia; third shows elongation of ovarian region and beginning of carpillary differentiation; fourth shows outline of central carpillary tissue clearly marked and (in disk type) commencement of equatorial thickening of wall; fifth shows young ovary in essentially its final shape. Dotted lines represent course of vascular bundles. From the fourth stage shown here, the three shape types begin to be clearly distinguishable. Outlines at extreme right show mature fruit shape which develops from each of these primordial series.

stand the mechanism by which a particular gene controls the development of a particular character. A necessary preliminary to such a study, particularly in quantitative traits, is a knowledge of the

stages, from inception to maturity, which are traversed by the structure displaying the traits in question. It is important, for example, to measure the shape of an organ in its various developmental stages, and to determine from microscopical observation those differences in the extent and localization of growth which are responsible for the shape ultimately produced. Particularly favorable material for a study of this sort is provided by our squash lines, and the present paper sets forth the results of an investigation of the development of the fruit, from floral primordium to maturity, in pure lines of *Cucurbita pepo* which differ markedly in fruit shape, and in which the differences have been subjected to genetic analysis. The specific effect upon development of various genotypes for shape can in this way be traced.

Plants were used from fifteen lines which have been inbred for from six to thirteen years, and which now breed very true for all their characters. These include three main shape types: the spherical, in which the length and width of the fruit are essentially equal at maturity; the elongate, in which the length is much greater than the width; and the disk, in which the width is much greater than the length. These are shown in outline in the right-hand column of fig. 1. Intermediates of all sorts may occur. The methods used in determining and plotting shape indices have been described in a previous paper (see footnote 2).

Stem tips from which most of the leaf primordia had been removed were killed in formol-acetic-alcohol, imbedded in paraffin, and sectioned for study of the earliest stages in ovary development. After the ovary primordia had reached a length of a few millimeters and could be handled separately, they were removed individually and killed and imbedded. Sections, both longitudinal and transverse, were made of all the stages in all the important lines. For late ovary and for fruit stages, macroscopic studies were made of the entire structure, and tissues from various portions were examined microscopically.

Results

The morphology and development of the pistillate flower of the Cucurbitaceae have been studied by many botanists, and a general survey of this subject for the family as a whole is well presented by

KIRKWOOD.³ Results from the material studied by us were found to conform closely to those obtained by previous workers, and are outlined diagrammatically in fig. 1, where the earliest stages are shown at the left and the progressively older ones toward the right. The rudiment of a pistillate flower first appears as a rounded swelling just below the growing tip of the stem. This swelling soon becomes broadened and flattened at its tip, and from the edge of the platform thus produced arise five prominences, the sepal primordia. Inside these and alternating with them soon appear five others, the petal primordia; and following these a circle of staminodia, which soon cease growth and are inconspicuous at anthesis. Meanwhile the tissue at the edge of the platform from which these circles spring elongates somewhat, producing a shallow concavity. At each of three points (sometimes four or five) on the inner wall of this cavity appears a ridge, and these ridges grow inward toward the center of the cavity, where they meet. These are the carpillary ridges, and along their recurved edges, from the base of the cavity to a point just below the level of the perianth primordia, develop the complex placentae where the ovules are ultimately borne. The ridges extend above this level and later fuse to form the style.

The morphology of the inferior ovary which is thus produced has been a matter of debate. Some botanists hold that the carpels give rise only to the stigmas, style, and upper portions of the ovary, the lower region being formed entirely from the floral axis. Others believe that the carpels constitute the entire ovuliferous tissue, lying with their backs to the ovary wall and bearing the ovules on their inrolled margins. The ovary wall itself is thought by some to be a downward prolongation of the perianth circles, but by others is regarded as an upward extension of a hollowed receptacle, to the inner face of which the carpels have become fused. The latter view is probably the most widely accepted today, and, at least for purposes of description, is adopted here. Certainly in many cases a rather definite line is visible, in early stages of the ovary, between an inner tissue, clearly continuous with the carpillary folds and consisting chiefly of cells elongated longitudinally, and an outer layer

³ KIRKWOOD, J. E., The comparative embryology of the Cucurbitaceae. Bull. New York Bot. Gard. 3:313-402. 1904.

in which most of the cells are elongated in a tangential direction. At a very early stage, when the first perianth primordia are developing, the procambial strands for ten vascular bundles appear, running up from the peduncle into the floral rudiment. These later branch repeatedly, but the ring of bundles thus formed remains relatively close to the outer surface of the ovary. These strands arise from a plexus of bundles at the base of the flower, where it joins the peduncle. Several definite layers may thus be recognized in the wall of the ovary and fruit: (1) the epidermis; (2) the cortical layer of the receptacle, relatively narrow; (3) the ring of vascular bundles; (4) the medullary layer of the receptacle, relatively wide; (5) the sterile outer layer of carpillary tissue; and (6) the central ovuliferous carpillary mass.

In developing ovaries of all the types studied, all these component layers are present, but the ovaries themselves differ markedly in shape. Indeed, the most striking fact which a comparative study of the various types discloses is that from almost the very beginning of differentiation each shape type is clearly distinguishable. In the gross differences in relative length and width of the ovary which determine these shape differences, however, the specific tissue layers do not share equally, their development relative to one another varying from type to type. Areas of localized growth also play an important part in the ultimate shape produced. Growth in such an organ as this is not a generalized phenomenon, therefore, affecting the organ uniformly and as a whole; but is a much more complex affair, and seems to be governed, at least to a certain extent, by factors which control particular processes or particular regions. The importance of this concept in problems of morphogenesis, particularly those concerned with the effect of genetic factors upon growth and form, is obvious.

A description of the developmental history of the more important shape types in squash fruits well illustrates this phenomenon of differential growth.

In all the shape types the very earliest stages are similar, but from a point soon after the floral primordium becomes flattened and begins to produce rudiments of the floral circles around its margin, differences begin to appear. Elongation of the tissue immediately

below the perianth rudiments, which carries them up above the floor of the cavity, proceeds at relatively different rates, in comparison with the growth in width, in the different types. In forms which will produce essentially spherical ovaries and fruits, this elongation is not markedly greater at first than the growth of the whole primordium in width, so that the structure from the tip of the peduncle to the base of the perianth rudiments is, throughout these early stages, not very much longer than wide. In the elongate types, however, growth in the longitudinal dimension is relatively much more rapid and lateral growth less so, so that from a very early stage the ovary primordium is much longer than wide. As development proceeds, growth in width keeps pace, proportionally, with growth in length, so that the ratio between these dimensions remains essentially constant.

It is noteworthy, however, that between these two types (spherical and elongate) there is almost no difference, at corresponding stages, in the width of the ovary wall (receptacular cortex, vascular cylinder, and pith), but that almost from the first the essential shape difference is one which concerns only the central carpillary tissue, which will ultimately develop into the seed cavity. Factors for shape in these types produce their effect by controlling the dimensions of this region, which, whatever its shape, is surrounded by a wall of uniform width.

Aside from this difference in the proportions of the ovarian cavity, the elongate types differ in another important particular from the spherical ones, namely, in the production of a sterile "neck" at the base of the ovary, between the cavity and the flower stalk (fig. 1 *A*). This begins its development after the elongation of the carpillary zone has started but while the whole primordium is still in a very early stage and is only a few millimeters long. The elongation of the neck carries up the rest of the primordium for some distance, but after an early period of rapid growth the ratio of neck length to the length of the rest of the ovary remains essentially constant. Growth in length of the neck is carried on for a time through the continued meristematic activity of the region at its base, nearest the peduncle, although in later stages growth is chiefly interstitial. The neck is clearly a part of the ovary and not a

continuation of the stalk, for the tissues of these two organs are readily distinguishable from one another and there is also a definite plexus of vascular bundles which separates them. The sterile outer layer of carpillary tissue often runs down for a considerable distance into the neck, forming a V-shaped area which can well be seen in longitudinal sections of mature fruits of this type.

The relative length of neck varies considerably in different lines. In disks and true spheres it is absent, in elongate spheres or fruits of the "Fordhook" type it is short, and in true elongates it may even exceed the ovarian cavity in length. Its development seems to be correlated to some extent with the shape of the ovary proper, but at least in certain cases it may be inherited independently of ovary shape, as is shown by the occurrence of "jug" fruits in the descendants of crosses between disks and elongates. Here the ovarian part of the fruit is broad and flat, but is provided with a definite neck or "handle" at the base.

The disk or flat type of ovary and fruit differs from the sphere and the elongate partly by the shape of the ovarian cavity or central carpillary tissue, which is relatively much shorter and broader in these forms. This manifests itself early in the development of the primordium by the greater width of the platform and the comparatively small amount of elongation. In one of our lines of disks, grown from a Mandan Indian race of squashes, this flattening of the ovarian cavity is the only respect in which the ovary and fruit differ from the more elongate types, for in this race the ovary wall (and later the pericarp) is of essentially uniform thickness at all points, and the ovary thus resembles that which would be produced by the marked flattening of a spherical type.

The other disk lines studied, however, show not only this flattening of the ovarian cavity, but, in addition, a marked increase in the relative thickness of the ovary wall, particularly in the equatorial region, which gives rise to the scallop or border so characteristic of the fruit of these types. This thickening does not affect all the wall layers, but is confined to the inner one consisting of the receptacular pith and the outer carpillary zone, the distinction between which is here difficult to observe. It does not affect the cortex (that portion outside the vascular ring), which is no wider here than in corre-

sponding stages of spherical and elongate types. This localized lateral growth makes its appearance at an early period in the development of the flower, about the time that the ovarian cavity becomes closed. It is initiated by numerous and somewhat regular tangential divisions, which soon expand the ovary wall in the equatorial region to a thickness considerably greater than that above and below; and this relative thickness, thus early attained, persists throughout the development of the flower and fruit. The position and the degree of development of this zone vary considerably in different races. In some it is strongly marked, producing a much flattened fruit type, but in others it is far less conspicuous. It may be considerably nearer one pole than the other, producing in consequence a disk with the appearance of either a reflexed or an upturned border. It is usually more strongly developed at certain points than at others, producing marked teeth or ribs. This equatorial thickening is sometimes found in squashes in which the ovarian cavity is clearly of the spherical or the elongate type, but here it is rarely as well developed or sharply localized as in disk forms. Undoubtedly, however, as in the case of the neck, the development of this tissue is a trait which may be inherited, in part, quite independently of the character of the rest of the ovary.

Each shape type is distinguishable from a very early stage, certainly from a period when the ovary is no more than 1 mm. in width. The shape of the ovary during its early development is somewhat more elongate than that which it assumes in the flower and fruit, doubtless because of its more pronounced growth in width at the time when the ovules mature. The remarkable fact, however, is that in its major aspects fruit shape is established at an extremely early period of development, when the volume of the ovary primordium is often less than one-millionth of the volume of the fruit at maturity.

No difference in size or shape of cells could be observed between the various squash types studied. In every case the cells of the early primordium are small and uniform in size. As development and differentiation progress, the cells of certain tissues, such as the pith of the receptacle, increase in size more rapidly than others, and as the ovary grows larger its component cells tend gradually to become

somewhat larger; although this increase is by no means proportional to the enlargement of the organ as a whole, which is chiefly effected by increase in cell number rather than by cell size. In the ripening fruit the increase in cell size is more conspicuous than in the early stages. In all cells, the actual volume of the nucleus changes relatively little during ovary development. It is noteworthy that the differences in relative dimensions observed in the various shape types are not correlated with differences in the relative dimensions of the cells composing them.

Discussion

Such a developmental study as this tends to emphasize certain general conclusions as to the determination of form. First, the form of the organ is established at a very early stage in its development, and as growth proceeds this shape is maintained, the relation of the various dimensions to one another remaining essentially constant (at least under similar environments). This must be due to a factor or factors controlling correlation of growth. Shape obviously cannot be merely a resultant of the extent of growth in a single dimension (such as length) in proportion to the total increase in bulk of the organ, since the ratio of a single dimension to total volume changes profoundly with increase in size, whereas shape does not change. Through some mechanism, be it the distribution of hormones or specific organ-forming substances, or the establishment of a gradient of some sort in a specific protoplasmic base, growth in one dimension or region is definitely correlated with growth elsewhere. Whatever the mechanism by which these shape differences are brought about, we can say that, in the present case at least, it involves control over two quite different processes or aspects of growth: (1) the plane of cell division in growing tissues, and (2) the localization of growth in various parts of the developing organ.

The shape differences in the ovary proper, in this species, result from the fact that growth in one dimension (length) is relatively more rapid or less rapid than growth in the other dimension (width). Since the cells themselves are of essentially the same size and shape in the various shape types, these differences are evidently due to more frequent cell divisions in one plane than in the other. What-

ever it is that controls the polarity of the cells, and thus determines the orientation of the equatorial plate at mitosis, determines to a very considerable extent the organic form which is ultimately produced. Cell polarity may be said to be one of the fundamental problems of morphology.

The second aspect of growth control is also of importance, because growth is not uniformly distributed through the tissues of a developing organ, but is often somewhat localized and proceeds at different rates in different regions, with a marked effect on the form produced. The fact that in certain lines of squashes an active growing region between the base of the ovary and the cavity is responsible for the development of a conspicuous neck in these types, and the rapid growth of the inner ovary wall in the equatorial region of most disk types (which produces the border so characteristic of them) are examples of such localized growth.

The shapes resulting from growth thus controlled and localized are constant within lines which are genetically pure, and breeding investigations have shown that they are transmitted in inheritance in a manner essentially Mendelian and similar to that shown by other characters of the plant. It is therefore fair to conclude that the specific differences in growth correlation which result in these shape differences are controlled by specific genes.

Summary

1. The inheritance of fruit shape in *Cucurbita pepo* may be ascribed to the operation of Mendelian factors.
2. In inbred and genetically pure lines showing the three main shapes of fruit (elongate, spherical, and disk), comparative studies were made of the developmental history of the various shape types from the earliest floral primordium to maturity.
3. From a very early stage in differentiation, when the ovary primordium is not more than one-millionth of the volume of the mature fruit, each of the various shape types is clearly distinguishable.
4. Much of the difference between the types, especially between the elongate and the spherical, was found to be due to shape differences involving only the central carpillary tissue (later the seed

cavity) and not the wall of the ovary and fruit, the latter maintaining an essentially constant width in fruits of diverse shapes.

5. In most of the disk types, however, not only is the seed cavity relatively short and broad, but there is also a localized thickening of the wall in the equatorial zone (involving only the inner wall layers) which results in a still flatter shape for fruits of this type.

6. In certain lines a sterile and relatively slender neck is developed at the base of the ovary.

7. The various fruit shapes are essentially similar in the size and shape of their component cells.

8. The factors determining shape are evidently those which govern growth correlation. They operate by controlling: (1) cell polarity, and thus the plane of cell division; and (2) the localization of growth in particular regions.

BARNARD COLLEGE, COLUMBIA UNIVERSITY AND
CONNECTICUT AGRICULTURAL COLLEGE
STORRS, CONN.

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COMPOSITION OF AVOCADO TREES IN RELATION TO CHLOROSIS AND TIP-BURN¹

A. R. C. HAAS

The sensitivity of avocado trees to low temperatures has made it necessary that the trees be planted on slopes within short distances from the ocean, where suitable temperatures prevail. Such a restriction upon plantings, while eliminating the frost hazard, in many cases has not given suitable soil conditions for the growth of the trees. On the slopes extremely shallow soils are often encountered at the higher elevations, and frequently such hill sides show extensive outcroppings of limestone. In some coastal districts the water available for irrigation purposes contains considerable amounts of salts, such as chlorides and sulphates. The trees in such districts are subject to strong ocean winds, which although laden with moisture are an unknown factor in the desiccation of the leaves. HAAS and REED (2) have pointed out that dry winds may cause a temporary accumulation of salts within citrus leaves as a result of excessive transpiration, but the effect is not known as yet in regard to moist winds of high velocity. Chlorination of the irrigation water reservoirs in coastal districts is a common practice, and nothing is known in regard to the effect of such additions of chlorine upon the chlorine content of the irrigation water as it is delivered to the grower.

Heretofore the burning of avocado leaves has been ascribed to too long an interval between irrigations. This conclusion has been due to the fact that this tree is usually shallow rooted, and does not do well under extremely dry atmospheric conditions or under intense heat, but thrives best in the more humid coastal districts. Notwithstanding an adequate irrigation program, the leaves of avocado trees in many districts show tip-burn during late summer or early autumn, and progressive marginal burning² during the winter. Many if not all of these burned leaves fall the following spring as the new growth

¹ Paper no. 192, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

² The term tip-burn as commonly used by growers may indicate any stage of leaf burn that originates at the leaf tip.

appears, with the result that the sun's rays have full access to the branches and trunk. Young shoots may be killed as a result of sun-burn, while the bark of older branches or the trunk may become dry and corky.

Very little is known in regard to the nutrition of avocado trees; in fact, some growers fertilize their trees very little, if at all, largely because of the difficulty of noting any relationship between fruiting and soil fertilization. The practices employed at present, in view of the lack of scientific data, are based largely on the results obtained in the culture of citrus. The present knowledge regarding cultural practices, and the frost resistance of the different varieties of avocado, has been well summarized by RYERSON, JAFFA, and Goss (4). The present investigation is merely a beginning in the direction of an understanding of some of the difficulties encountered in avocado culture.

Many of the contoured slopes in the coastal districts show conspicuous outcroppings of limestone, so that the newly leveled soil above these outcroppings is frequently shallow and heavily charged with limestone. Newly planted trees in such locations may become chlorotic during the first season of growth or at any subsequent time. Applications of iron sulphate to the soil or injections of iron sulphate solution into the trees were found by THOMAS and HAAS (5) to be unsuccessful in overcoming the chlorosis, even temporarily, without injuring the trees. In such locations the generally accepted theory is that the trees are suffering from a faulty distribution of the iron supply within the tree. The function of iron is understood to be that of catalyzing the formation of chlorophyll, of which magnesium is a constituent. In chlorotic avocado leaves a reduced amount of chlorophyll is not necessarily accompanied by a reduction in the magnesium content of the leaves, as seen in table I. In such cases, however, the absorption of calcium by the leaves is excessive, so that the calcium present in relation to the magnesium is much greater than that occurring in normal leaves. The results obtained in table I for the calcium and magnesium content of chlorotic leaves do not necessarily mean that a definite ratio exists between calcium and magnesium in normal leaves, but rather that within a wide range of ratios the leaves may be expected to be normal.

In table II are given water-solubility data for the various inorganic constituents of dry avocado leaves. The ash of the soluble fraction represents relatively only a small part of the inorganic constituents of the dry matter. In the case of the chlorotic leaves the amount of ash of the water-insoluble portion is considerably larger than in the case of normal leaves. This is due to the large amount of insoluble calcium present. It is of interest to note the extremely low

TABLE I

EFFECT OF CHLOROSIS ON CALCIUM AND MAGNESIUM CONTENT OF AVOCADO LEAVES

| VARIETY | LOCALITY IN CALIFORNIA | CONDITION OF LEAVES | ASH AS PERCENTAGE OF DRY MATTER | PERCENTAGE ASH | | PERCENTAGE DRY MATTER | | Ca/Mg |
|---------------|------------------------|---------------------|---------------------------------|----------------|-------|-----------------------|-------|-------|
| | | | | Ca | Mg | Ca | Mg | |
| Fuerte..... | La Habra | Normal | 9.60 | 16.64 | 11.52 | 1.507 | 1.106 | 1.444 |
| Fuerte..... | Montebello | Leaf-burn | 9.52 | 19.76 | 11.41 | 1.881 | 1.085 | 1.733 |
| Puebla..... | La Habra | Normal | 9.61 | 18.49 | 10.37 | 1.778 | 0.997 | 1.783 |
| Fuerte..... | La Habra | Normal | 10.33 | 15.84 | 10.32 | 1.638 | 1.067 | 1.535 |
| Fuerte..... | Montebello | Leaf-burn | 9.23 | 18.18 | 9.86 | 1.679 | 0.910 | 1.845 |
| Puebla..... | La Habra | Normal | 9.53 | 18.25 | 9.54 | 1.739 | 0.909 | 1.913 |
| Fuerte..... | Brentwood | Normal | 8.36 | 15.56 | 8.37 | 1.300 | 0.699 | 1.859 |
| Fuerte..... | Culver City | Leaf-burn | 9.64 | 19.24 | 8.03 | 1.855 | 0.775 | 2.394 |
| Seedling..... | Brentwood | Leaf-burn | 9.48 | 17.43 | 7.70 | 1.653 | 0.730 | 2.264 |
| Fuerte..... | La Habra | Chlorotic | 9.04 | 16.78 | 5.90 | 1.517 | 0.534 | 2.841 |
| Puebla..... | La Habra | Chlorotic | 15.25 | 22.93 | 5.90 | 3.498 | 0.900 | 3.887 |
| Fuerte..... | La Habra | Chlorotic | 12.98 | 22.48 | 5.83 | 2.917 | 0.757 | 3.853 |
| Lyon..... | La Habra | Chlorotic | 8.36 | 19.20 | 5.36 | 1.606 | 0.449 | 3.577 |
| Lyon..... | La Habra | Chlorotic | 11.41 | 16.10 | 4.99 | 1.837 | 0.569 | 3.229 |
| Fuerte..... | La Habra | Chlorotic | 10.32 | 20.80 | 4.94 | 2.147 | 0.510 | 4.210 |
| Puebla..... | La Habra | Chlorotic | 18.63 | 25.97 | 4.77 | 4.840 | 0.889 | 5.444 |

water solubility in the case of the calcium of avocado leaves, as compared with the values given by HAAS (1) for citrus leaves. The calcium present in normal avocado leaves is considerably less than that present in citrus leaves. The magnesium content of avocado leaves, however, is from two to three times as great as that of citrus leaves. This large magnesium content may be of significance from the standpoint of the concentration of chlorophyll in avocado leaves and their capacity to manufacture carbohydrates, fats, and proteins. Practically all of the potassium is found in the water-soluble fraction. It is of interest to note the large concentration of potassium in the water-soluble fraction of the chlorotic leaves.

Table III shows the total phosphorus content of the dry matter

TABLE II
WATER-SOLUBILITY OF DRY MATTER OF MATURE AVOCADO LEAVES, EXPRESSED AS PERCENTAGE OF DRY MATTER

| VARIETY | LOCALITY IN CALIFORNIA | CONDITION OF LEAVES | ASH OF | CALCIUM | | MAGNESIUM | | POTASSIUM | | SODIUM | |
|-------------|------------------------|---------------------|--------|------------------|--------------------|---------------------------------|-----------|---------------------------------|-----------|---------------------------------|-----------|
| | | | | Soluble fraction | Insoluble fraction | Soluble as per centage of total | Insoluble | Soluble as per centage of total | Insoluble | Soluble as per centage of total | Insoluble |
| Fuerte..... | La Habra | Normal | 3.52 | 6.81 | 0.086 | 1.552 | 0.747 | 0.320 | 70.01 | 0.508 | 0.026 |
| Puebla..... | La Habra | Normal | 3.05 | 6.49 | 0.097 | 1.642 | 5.58 | 0.587 | 3.226 | 0.678 | 0.034 |
| Puebla..... | La Habra | Chlorotic | 4.24 | 14.45 | 0.099 | 4.755 | 2.04 | 0.605 | 0.375 | 65.73 | 1.544 |
| Puebla..... | La Habra | | | | | | | | | 0.112 | 0.077 |

of the leaves as determined by the magnesium nitrate method. The values for the most part are not very different from those found for citrus leaves.

Table IV gives the percentage of total nitrogen in the dry matter of the leaves of four varieties of avocado. In some cases the nitrogen content is equal to that found in citrus leaves. When an avocado leaf burns, it is not known what becomes of the nitrogen in the burned area, whether it remains in the burned portion, or passes back into the unburned portion and remains there, or passes back into the shoots for new distribution. However, any water-soluble nitrogen remaining in the burned portion is likely to be leached out,

TABLE III
PHOSPHORUS CONTENT OF AVOCADO LEAVES

| VARIETY | LOCALITY IN CALIFORNIA | CONDITION OF LEAVES | TOTAL PHOSPHORUS AS PERCENTAGE DRY MATTER |
|-------------|------------------------|---------------------|---|
| Fuerte..... | La Habra | Normal | 0.08 |
| Fuerte..... | La Habra | Normal | 0.24 |
| Fuerte..... | Montebello | Leaf-burn | 0.15 |
| Puebla..... | La Habra | Normal | 0.15 |
| Puebla..... | La Habra | Normal | 0.29 |
| Puebla..... | La Habra | Normal | 0.24 |
| Puebla..... | La Habra | Chlorotic | 0.19 |

especially with overhead irrigation, so that no comparison can be made between the nitrogen content of burned and that of normal leaves. Flowers of Puebla trees at Riverside, California, were collected and were separated from the flower stalks. The total nitrogen of the flowers was found to be 1.71 per cent of the dry matter. At present it is not known whether failure of avocado trees to set fruit may be in any way related to the nitrogen content of the flowers.

A high nitrogen content of a soil may become of very great importance when the soil solution contains considerable amounts of chlorides and sulphates. HAAS and THOMAS (3) have shown the toxic effect of sulphates on lemon trees to be much greater when the nitrate supply was inadequate. As was previously mentioned, many of the coastal and other avocado districts are irrigated with water containing considerable chloride or sulphate. The occurrence of tip-

burn has not always been exclusively associated with the use of such irrigation water; in fact, it has been found to occur where the irrigation water was of good quality. The burning of a small portion of the leaf tip alone may occur as a consequence of an inadequate water supply, as was shown in sand cultures which received a culture solution containing no chlorine. In this case the sand cultures were covered but were not kept sufficiently moist, and although provided with a drainage system, they showed no drainage water except at the time of the addition of large amounts of new nutrient every three to

TABLE IV
NITROGEN CONTENT OF AVOCADO LEAVES

| VARIETY | LOCALITY IN CALIFORNIA | CONDITION OF LEAVES | TOTAL N AS PERCENTAGE DRY MATTER |
|-------------|------------------------|---------------------|----------------------------------|
| Fuerte..... | La Habra | Normal | 2.52 |
| Fuerte..... | Box Springs | Normal | 1.87 |
| Fuerte..... | Riverside | Normal | 1.84 |
| Fuerte..... | Riverside | Leaf-burn | 1.80 |
| Fuerte..... | Riverside | Leaf-burn | 1.77 |
| Fuerte..... | Culver City | Leaf-burn | 1.76 |
| Fuerte..... | Lemona | Leaf-burn | 1.30 |
| Taft..... | Riverside | Normal | 1.98 |
| Taft..... | Riverside | Leaf-burn | 2.25 |
| Taft..... | Lemona | Leaf-burn | 2.20 |
| Puebla..... | Riverside | Leaf-burn | 2.07 |
| Lyon..... | La Habra | Normal | 2.53 |

four weeks. Such leaf-burn is not serious and is not the condition usually designated as tip-burn. In this latter condition one-fourth or more of the apical portion of the leaf may be brown and extremely desiccated, often with marginal burning proceeding farther toward the basal portion of the leaf. It may be mentioned that control cultures which were kept sufficiently moist were free from such tip-burn.

The causal factors producing this burned-leaf condition of the avocado tree, popularly called tip-burn, are best understood by comparing the analyses of normal avocado leaves (table V) with those of leaves affected with tip-burn (table VI). It is at once obvious that the leaves affected with tip-burn contain excessive amounts of total chlorine. In at least two of the cases examined, total sulphur may also be a contributing factor in bringing about

the burning of the leaves. It is of interest that in some cases very little sulphate was found in the leaves, even though the irrigation water contained a considerable amount.

TABLE V

TOTAL CHLORINE AND SULPHUR CONTENT OF MATURE, NORMAL AVOCADO LEAVES

| VARIETY | COLLECTED | LOCALITY IN CALIFORNIA | PERCENTAGE IN DRY MATTER | |
|-------------|-----------|------------------------|--------------------------|---------|
| | | | Total Cl | Total S |
| Fuerte..... | 3/16/26 | Brentwood | 0.33 | |
| Fuerte..... | 3/16/26 | La Habra | 0.32 | |
| Fuerte..... | 3/10/28 | Riverside | 0.09 | 0.25 |
| Puebla..... | 3/10/28 | La Habra | 0.21 | |
| Taft..... | 3/10/28 | Riverside | 0.17 | 0.29 |

TABLE VI

TOTAL CHLORINE AND SULPHUR CONTENT OF MATURE AVOCADO LEAVES
SHOWING TIP-BURN

| VARIETY | COLLECTED | LOCALITY IN CALIFORNIA | PERCENTAGE IN DRY MATTER | | IRRIGATION WATER* | |
|---------------|-----------|------------------------|--------------------------|---------|-------------------|-----------------|
| | | | Total Cl | Total S | Cl | SO ₄ |
| Fuerte..... | 1/25/28 | Culver City | 0.91 | | p. p. m. | p. p. m. |
| Fuerte..... | 3/24/26 | Culver City | 0.88 | | 269-305 | |
| Fuerte..... | 3/10/28 | Riverside | 0.91 | 0.31 | 269-305 | |
| Fuerte..... | 3/10/28 | Riverside | 0.93 | 0.37 | 195-213 | 140-254 |
| Fuerte..... | 4/16/26 | Montebello | 1.00 | | 195-213 | 140-254 |
| Fuerte..... | 3/29/26 | Montebello | 1.21 | 0.88 | 385 | 42 |
| Fuerte..... | 3/29/26 | Montebello | 1.13 | | | |
| Fuerte..... | 4/13/28 | Lemonia | 1.01 | | 52 | |
| Fuerte..... | 5/17/26 | Irvine | 0.80 | | | |
| Seedling..... | 3/16/26 | Brentwood | 1.34 | 1.13 | | |
| Puebla..... | 3/10/28 | Riverside | 1.01 | 0.22 | 195-213 | 140-254 |
| Taft..... | 3/10/28 | Riverside | 0.54 | 0.28 | 195-21 | 140-254 |
| Taft..... | 3/10/28 | Lemonia | 0.72 | 0.28 | 52 | 28-32 |

* The writer is indebted to the Department of Chemistry of the Citrus Experiment Station for a portion of these analyses.

The chlorine content of the leaves of the trees at Lemonia, California, is high in comparison with the low amount of chlorine in the irrigation water. In this case the water did not penetrate into the subsoil below the root zone. As root absorption and surface evaporation withdrew water from the soil, the chlorine that was added in the irrigation water could not escape into the subsoil, and conse-

quently accumulated in the moisture about the tree roots. The trees were given very little if any nitrogen, and as a consequence chlorine was absorbed in sufficient amounts to burn the leaves. In another of the coastal districts, in connection with studies on the growth of citrus trees, as conducted by FAWCETT and the writer, it was found that, although the irrigation water and the first two feet of soil were relatively free from chloride and sulphate, the third and fourth feet of soil were rather heavily charged with sulphate. It was found that chlorination of the irrigation water at the irrigation-district dam did not increase the chlorine content of the water upon its arrival at the groves.

A source of chlorine that makes its way into avocado groves is found in certain barnyard manures. In some districts the more saline water that is unsuited for tree culture is used in growing alfalfa, etc., for dairy purposes, with the result that such manure frequently finds its way into the groves. In a specific case the manure applied contained 23 pounds of chlorine in one ton of dried material. When this supply supplements an already somewhat saline irrigation water, the application may hasten the appearance of injury. In certain avocado groves where the trees were losing most of their leaves as a result of tip-burn, concentrations of chlorine (as determined from a one to five water extract of the soil) constituted one-third of the total solids obtained from such water extracts.

In the selection of sites which have been valued largely according to the degree of frost protection they afford, due consideration should be given also to the nature, depth, and drainage of the soil and subsoil, to the amounts of chloride and sulphate in the irrigation water, and to the nature of the materials used in the fertilization program.

Summary

1. The effect of lime-induced chlorosis on avocado trees is to raise the calcium-magnesium ratio.
2. The total and the water-soluble calcium of the dry matter of normal mature avocado leaves are considerably less than that of citrus leaves. The magnesium content of the dry matter of normal mature avocado leaves is two to three times that of normal mature citrus leaves. Chlorotic avocado leaves have been found to con-

tain large amounts of water-insoluble calcium and water-soluble potassium.

3. The total nitrogen and phosphorus contents of avocado leaves are approximately the same as those found in the leaves of citrus.

4. Tip-burn of avocado leaves has been found to be associated with a high chloride or sulphate content of the leaves.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

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Oogenesis and Fertilization in *Volvox*
Contributions from the Hull Botanical Laboratory 389
Caroline A. Lander
(With Plate XV)

Introduction

The recent taxonomic monograph of the Volvocales by PASCHER¹ suggested that a critical cytological study of some of the features in the life history might be worth while. A great deal of work has been done on *Volvox*, both by botanists and zoologists, but very little cytological work has been done with sectioned material.

Investigators have had difficulty in finding fertilization, and though it has been reported that sperms were seen in a boring motion, they have not been identified entering the egg. It is believed by some that they enter at the neck. No fusion stages have been reported. Although a study of fertilization was the chief object of this investigation, the invagination theory as presented by ZIMMERMANN² in 1925 was examined; and the formation of starch from the pyrenoids was traced through the life history.

ZIMMERMANN believes that as the asexual colony develops from the two to eight and on through the eleven successive divisions there is an arching, and gradually a hollow ball is formed. The pore enlarges and the colony turns inside out.

The formation of starch from the pyrenoids has been reported before in *Volvox*, and studied in detail in *Hydrodictyon* by TIMBERLAKE.³

Materials and methods

The material for this study was *Volvox globator*, collected in 1916 at Mud Lake, Ward, Colorado. It was killed in 0.5 per cent chromic

¹ PASCHER, A., Die Süßwasser-Flora. Volvocales. Jena: G. Fischer. 1927.

² ZIMMERMANN, W., Die Un geschlechtliche Entwicklung von *Volvox*. Die Naturwissenschaften. Berlin. 13: 1925.

³ TIMBERLAKE, H. G., Pyrenoid and starch formation in *Hydrodictyon*. Ann. Botany 15: 619-635. 1901.

acetic acid and imbedded in paraffin. Sections were cut at 3 and 5 μ and stained by two methods. The safranin, gentian violet method was best for showing the formation of starch from the pyrenoid, the pyrenoid staining red and the starch blue. The sections were allowed to remain in the safranin over night, counterstained with gentian violet from one to ten minutes, according to the structures desired, and destained with clove oil. The other method used, Haidenhain's iron-alum haematoxylin, showed the nuclear structure better.

Investigation

EGG DEVELOPMENT

The cells of a colony are all apparently alike, each connected with its neighbor by protoplasmic strands, and containing a nucleus, an eye spot, and a chromatophore with a pyrenoid imbedded in it.

The first difference noticed in a reproductive primordium, or gonidium, whether asexual or sexual, is an increase in size (fig. 1). When a reproductive cell reaches about twice the diameter of a vegetative cell, the protoplasm has become more abundant and the sections stain more deeply. The outline becomes broadly pear-shaped or almost roundly triangular in cross-section. The cilia disappear before the increase in size takes place, or as it is taking place; it was not determined which. Even at this early stage the pyrenoid divides, so that such a cell contains two pyrenoids. From this stage the increase in size is very rapid, and is accompanied by an increase in the size of the nucleus and nucleolus, and a rapid increase in the number of pyrenoids (figs. 2, 3).

When the diameter has increased to six times the diameter of the original cell, the asexual gonidium and egg cell are indistinguishable; both are more broadly oval-shaped and contain six to twelve pyrenoids (fig. 3). From this point on, however, they differ somewhat. The nucleus of the egg moves to the center of the cell and enlarges (fig. 4). At this stage, the cell which is to form a new colony divides longitudinally, and thus is easily distinguished from one which is to form an egg.

After the nucleus of the egg has reached the center, its increase in diameter does not keep pace with the increase in protoplasmic content; but the pyrenoids continue to increase in number until the

egg is mature. The amount of food material increases up to the time of fertilization. At this time the egg has become rounded off within the parent cell, which is really an oogonium. At the time of fertilization it is a roundly pear-shaped body with a large nucleus containing a prominent nucleolus and rather scanty chromatin. Its cytoplasm is filled with various food materials, principally oil, volutin; and near the periphery, a zone of pyrenoids producing an abundance of starch, in the form of disk-shaped flakes surrounding each pyrenoid (fig. 5). The eggs of a colony seem to be of about the same size.

INVAGINATION OF DAUGHTER COLONY

Even after the first division of the asexual gonidium, in the two-celled stage there is an arching and the two cells seem to spread apart at the pore (fig. 6). This arching continues until in the eight-celled stage the axis is at right angles to the original axis (fig. 8). The nuclei remain toward the inside of the colony. This condition persists until eleven or more successive divisions have taken place, and then the colony turns inside out through the pore which has widened greatly (fig. 10). After this invagination, which causes the nuclei to be toward the outside, the cilia are formed.

PYRENOID AND STARCH

Starch is formed from the pyrenoids. Even in the vegetative cells a zone of starch is found around each one (figs. 1, 2, 3, 6, 7). In each stage of the division of the daughter colony (figs. 6, 7, 8, 9, 10), and all stages of the egg up through the mature zygote (figs. 1, 2, 3, 4, 5, 13, 17) slabs of starch are cut off. Some pyrenoids show just one piece of starch lying on its outer edge, but more show two, three, or even five or six pieces. These may form a definite zone around the pyrenoid, or they may finally come to lie in various positions near the pyrenoid (fig. 17). There may be a clear area between this zone and the edge of the pyrenoid, and the area sometimes takes a faint stain, as if starch were forming there in a dilute form. Sometimes two rings of starch are cut off around the pyrenoid. Because of the various pieces cut off the pyrenoids present peculiar shapes. The pyrenoid is most active in producing starch during the final

stages in the development of the egg before fertilization, and after fertilization until the zygote becomes vacuolate (figs. 4, 5, 13).

FERTILIZATION

The evidence shows that the sperm enters the egg from the interior of the colony. In many cases sperms were found lying on the oogonium wall on the side of the egg (fig. 11). One sperm was seen starting to penetrate the oogonium wall. The cilia could not be identified, and the nucleus was larger than those of the sperms in the mature sperm colony. The sperm enters, ciliated end first, through a pore in the oogonium wall (fig. 12). At the point of entrance there is a receptive spot or wider area between the cytoplasm of the egg and the outside of the oogonium wall (fig. 12). It appeared as if the cytoplasm of the egg had shrunk back to form the receptive spot, or some cytoplasm may have been discharged, as in *Oedogonium* and other algae.

As soon as the sperm enters, the border of the egg, which up to this time is merely a plasma membrane, begins to thicken and to stain like cellulose. For a while the sperm nucleus is hard to identify, probably on account of the dense mass of food material, for hundreds of eggs were examined at this stage, and the nucleus was not identified until it had almost reached the nucleus of the egg. The two nuclei come together with their nuclear membranes intact (fig. 13). Although the sperm nucleus has increased greatly in size since it entered the egg, it is slightly smaller than that of the egg, but their nucleoli are of about the same size. The membrane of the egg nucleus now becomes thinner at the point of contact, the membrane of the sperm nucleus breaks (fig. 14), and its nucleolus and the chromatin pass into the egg nucleus. The nucleoli remain separate (fig. 15), but the behavior of the two chromatin masses was not traced, except to note that in the resting zygote only one chromatin mass could be identified (fig. 17).

Discussion

This investigation, although brief, is sufficient to warrant the conclusion that ZIMMERMANN is right in his invagination theory. If the nuclei and chromatophores change places in the cell, as the older investigators reported, one ought to be able to find the nuclei in the

process of changing; but this I was unable to do. In all the divisions of the asexual colony up to the hundreds of cell stage, where there are too many to count, the nuclei are inward and no cilia have been formed. Mature colonies are found with the nuclei outward and the cilia present.

A number of stages identical with those of ZIMMERMANN were found (figs. 6, 7, 8, 9, 10). These may be due to the fixative, however, for the majority of the colonies, even the mature ones, take peculiar shapes caused by the fixation. On the other hand, ZIMMERMANN's photograph of the living colonies in the act of invagination is sufficient proof that this behavior occurs.

The starch formed from the pyrenoid has been noticed before in the Chicago laboratory in the egg stage; but it has not before been thoroughly traced through the life history or studied in detail. The present work confirms in *Volvox*, from the morphological standpoint, the work of TIMBERLAKE on the pyrenoid and starch formation in *Hydrodictyon*.

Other investigators are right in finding that fertilization is very difficult to identify. Hundreds of eggs were examined and only a few stages found. But without doubt the material showed many sperms closely applied to the oogonium wall, and in several cases the sperm nucleus was seen within the egg (figs. 13, 14). The pore for the entrance of the sperm and the receptive spot behind it were identified (figs. 11, 12). This does not support the suggestion of OLTMANNS⁴ that the sperm enters at the neck of the egg. As yet no stages have been found from the time the sperm enters until the nucleus almost comes in contact with the egg nucleus. This present investigation shows the fusion stages (figs. 13, 14, 15, 16).

Summary

I. Starch is formed from the pyrenoids in the vegetative cells, in all stages of the division of the asexual colony, in all stages of the egg before and after fertilization, and in the mature zygote. It is most actively formed in the mature egg and in the early stages after fertilization.

⁴ OLTMANNS, T., Morphologie und Biologie der Algen. II. Jena: G. Fischer. 1922-1923.

2. The nuclei of the daughter colony cells remain on the inward side of the cell, but as the colony matures the pore enlarges and the colony turns inside out through the enlarged pore.

3. Sperms enter the side of the egg from the inside of the colony through a pore in the oogonium wall, behind which is a receptive spot. The sperm nucleus increases greatly in size and comes in contact with the egg nucleus. The nuclear membranes of the egg and sperm dissolve at the point of contact, and the sperm nucleolus and its chromatin mass enter the egg nucleus. In the mature zygote the chromatin masses have united.

I am indebted to Professor CHARLES J. CHAMBERLAIN for suggesting the problem of fertilization of *Volvox*, which is the real aim of this investigation; and also to Professor W. J. G. LAND for valuable and kindly advice and encouragement during the progress of the work.

HOOD COLLEGE
FREDERICK, MD.

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EXPLANATION OF PLATE XV

Figures were drawn with camera lucida; reduced magnification $\times 856$, except fig. 10, which is $\times 486$.

FIGS. 1-3.—Very early stages of egg gonidium.

FIG. 4.—Young egg.

FIG. 5.—Mature egg at time of fertilization; *p*, pyrenoid surrounded by starch.

FIGS. 6-9.—Asexual colony, stages of development.

FIG. 10.—Asexual colony, beginning of invagination.

FIG. 11.—Mature egg and sperm (*s*).

FIG. 12.—Mature egg showing pore.

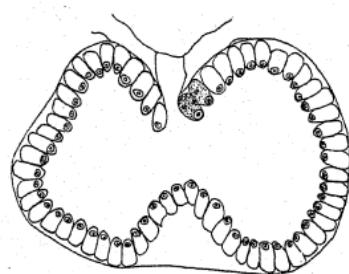
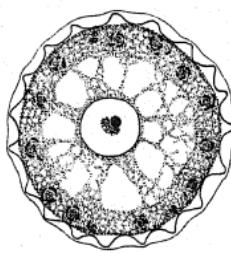
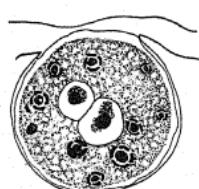
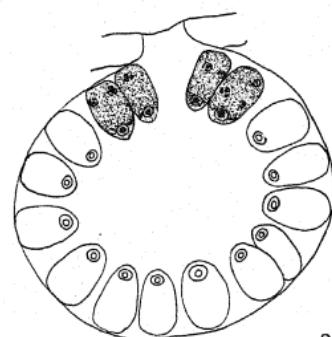
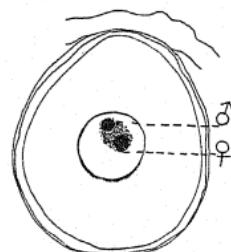
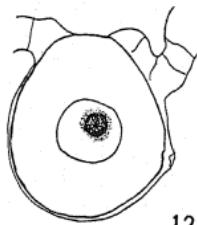
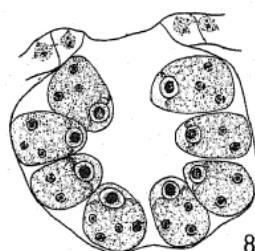
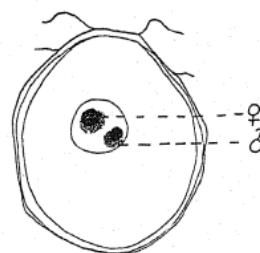
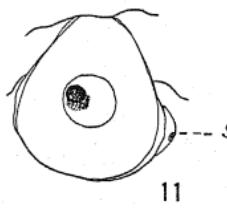
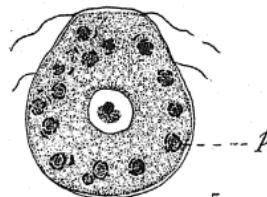
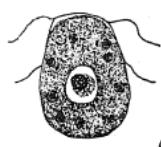
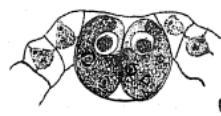
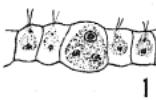
FIG. 13.—Fertilization, sperm and egg nuclei in contact.

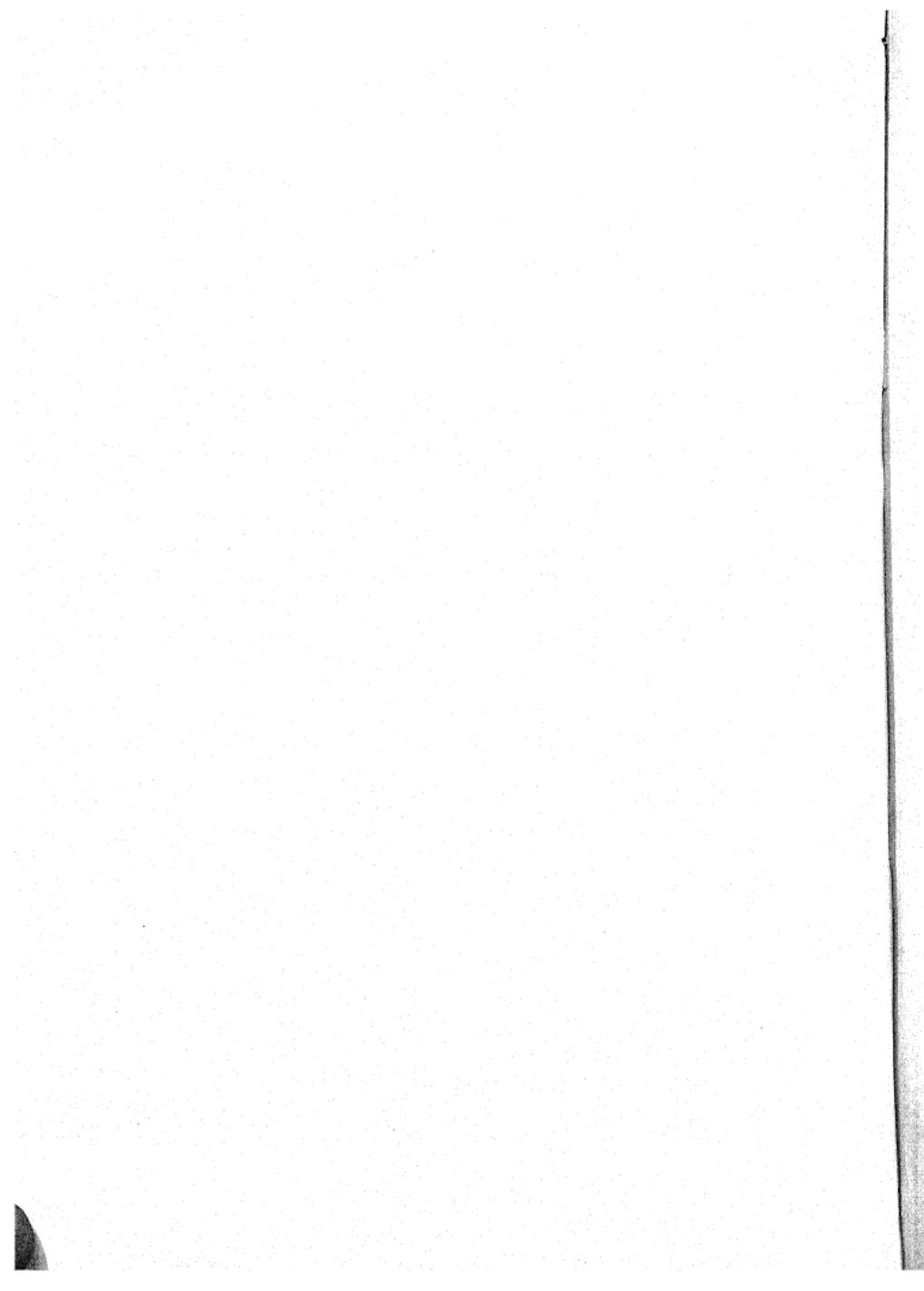
FIG. 14.—Fertilization, wall broken between sperm and egg nuclei.

FIG. 15.—Fertilization, two nuclei and chromatin masses in egg nucleus; female nucleus, male nuclei.

FIG. 16.—Fertilization, two nuclei and one chromatin mass in egg nucleus.

FIG. 17.—Mature zygote.





CURRENT LITERATURE

BOOK REVIEWS

North American cup-fungi

For many years the study of the section Operculates of the order Pezizales (a section often referred to as the operculate cup-fungi) has been handicapped for American students by the absence of a monograph of that group. SEAVER has recently published a volume¹ which should remedy this situation. In his own words, it represents "a summary of our knowledge of the group which has accumulated after many years of more or less intermittent observation and study." Pages 3-35 are occupied with preliminary discussions, accompanied by illustrative figures, upon phylogeny, gross morphology, origin of the apothecium, ascospore formation, alternation of generations, heterothallism and homothallism, spore discharge, heliotropism or phototropism, dehiscence of the ascus, significance of the mode of dehiscence, eccentricity of the ascostome, asexual reproduction, ecology, pyrophilous cup-fungi, coprophilous cup-fungi, spore germination, viability of the spores, mycophagy, classification, geographical distribution, and nomenclature.

The Operculates are taken to consist of two families, namely, the Pezizaceae (by much the larger group, and including, for the range treated, 39 genera, among them the well known *Peziza*) and the Elvelaceae (including, for the range treated, only 5 genera, the best known of which to the average student is doubtless *Morchella*). Something of the great confusion that has arisen in the literature of the subject may be seen from the large number of synonyms and names of doubtful status that are listed. Thus, to take only a few examples, for the genus *Patella* (p. 156) 14 synonyms are given; for *P. thelebooides* (p. 170) 16 synonyms; for *Bulgaria melastoma* (p. 197) 16 synonyms; for *Morchella hybrida* (p. 241) 15 synonyms; and, as against 20 accepted species of *Peziza* (pp. 219-221), there are 301 names indexed as synonymous (a majority going to other genera) or of dubious significance.

As might be expected in a pioneer work of this type, the author has found it necessary to propose many new species and to introduce various new combinations. In all, some 366 species and 4 varieties are maintained. The descriptions are in English. These are followed by the type locality, distribution, list of illustrations in literature, and list of *exsiccati* in herbaria. Generally speaking, the so-called American Code is followed. There are numerous plates, most of them half-tones, and these are remarkable for their excellence.

¹ SEAVER, F. J., The North American cup-fungi (Operculates). 8vo. pp. vii+284. *pls.* 45. *figs.* 15. Published by the author, New York City. 1928.

Toward the end of the book an intimation is given that a second volume may eventually be published, to deal with the Inoperculates. It is to be hoped that such will be the case, and that it will conform to the same high standards attained throughout the present volume.—E. E. SHERFF.

NOTES FOR STUDENTS

Mitogenic rays.—In a recent issue of this journal² there appeared a review by R. O. EARL of a paper by GUTTENBERG criticizing the so-called "mitogenic ray" theory of GURWITSCH. It is perhaps proper to call attention to certain points in this review. It appears that both GUTTENBERG and EARL have based their criticisms, not on the original papers, but upon GURWITSCH's recent review of his work,³ which, it must be acknowledged, is badly written from the western point of view and often misleading.

In the first place, EARL says, presumably on the authority of GUTTENBERG, that "these rays are claimed to be emitted from actively dividing root-tip cells and to induce mitosis in neighboring roots." It should be pointed out that GURWITSCH has never made such a claim; on the contrary, his narcosis experiments have demonstrated that the supposed ray is emitted not from such cells but from the base of the root. According to GURWITSCH's theory, a root does not emit these rays because it is dividing actively, but divides because the tissues (presumably leptome) at its base are emitting these rays. GURWITSCH considers the root tip the conductor but not the source of the ray. Moreover, he has never claimed that such a root would "induce mitosis in neighboring roots," unless the region of elongation of this neighboring root lies on a prolongation of the axis of the inducing root, a condition seldom met with in nature. Also, GURWITSCH and his co-workers have used as "sources" of the ray not only root tips of *Allium*, *Pisum*, and *Helianthus*, but also stem tips, cotyledons, and leaf vein tips of *Helianthus*, macerations of *Allium* bulbs, potato tuber leptome, yeast cells (both living and macerated), frog and axolotl eggs, the brains of tadpoles and axolotl embryos (both living and macerated), mixtures of haemoglobin from horse blood with serum from frog blood, arterial blood of frogs, etc., as well as the mercury vapor arc; and have used as detectors (induced organs) not only root tips of *Pisum* and *Allium* but also yeast cells, frog cornea, and other materials. To say that these rays emanate from root tips is true but misleading. GURWITSCH considers that they emanate from some embryonic tissues at periods of active metabolism (the emanation is suppressed under anaesthesia), but more especially from certain non-embryonic tissues. They can be produced in-vitro by mixing the extracted enzyme (mitotase), assumed to be a peroxidase, with some widely distributed oxidizable substance (mitotin), a behavior quite comparable with that of HARVEY'S "luciferase" and "luciferin," or to the materials

² Bot. GAZ. 86:119-120. 1928.

³ GURWITSCH, A., Das Problem der Zellteilung physiologisch betrachtet. Berlin: Verlag Julius Springer. 1926.

apparently involved in the emanation of ultra-violet light from cod-liver oil. The use of potato tuber leptome as a source is especially interesting in view of its possible relation to the "leptome hormone" of HABERLANDT and LAMPRECHT. Both EARL and GUTTENBERG seem, if they have known at all of most of this work, to have ignored it.

Further on, after mentioning very briefly and uncritically the general features of GURWITSCH's technique, EARL continues, again apparently relying on GUTTENBERG:

after at least twenty minutes the vertically placed tip (induced organ) was sectioned longitudinally, when many more mitoses were found on the side nearest the horizontal tip (inducing organ) than on that farthest from it. . . . A point of note is made regarding median sections. Here many more cells are seen than in tangential sections and so more cases of division. Thus with higher totals greater deviations in number are to be expected.

No cognizance is taken of the two- to three-hour lag period required between exposure and the time of maximum observable effect as determined by GURWITSCH.

EARL also states:

GUTTENBERG first points out the inadequacy of GURWITSCH's own data for a critical examination of the problem. The zones studied were not exactly indicated nor were the actual counts given in any complete or thorough manner. Figures were given in percentage of increase or numerical differences.

This is true of GURWITSCH's book, but not of the individual articles published from 1910 to date.⁴ In most of these periodic papers, especially the earlier ones on which the theory was based, a careful record of actual counts on the "induced" and "non-induced" sides of the induced organ is given, and it is only in the briefer review that percentage differences alone are given. Moreover WAGNER, in verifying GURWITSCH's results, has insured against any possible error in determining the relative positions of induced and inducing root by fixing both roots *in situ*, imbedding in the same block, and sectioning them together. And further, GURWITSCH has controlled each series of experiments against a blank root exposed to a capillary tube filled with water and placed in the same relative position as the inducing root, another point which has apparently escaped his critics.

It seems strange that not one of those working with GURWITSCH (ANIKIN, BARON, FRANK, L. GURWITSCH, N. GURWITSCH, KISLIAK-STATKEWITSCH, RAWIN, RUSINOFF, SALKIND, or SORIN) has noticed the errors which seem so glaring to GUTTENBERG and EARL. The work has been verified in Prague under the direction of NĚMEC (WAGNER) and by MAGROU in France. It seems very un-

⁴ Arch. Entwick. 30:133-193. 1910; 32:447-471. 1911; 52:167-187. 1922; 100: 11-40. 1924; 101:53. 1924; 103:68-79. 1924; 103:490-498. 1924; 104:109-115. 1925; 105:470-472. 1925; 105:473-474. 1925; 107:829-832. 1926; 109:362-. 1927, as well as numerous articles in the same journal by other workers.

fortunate that none of those who have criticized this work (EARL, LANDAUER, GUTTENBERG, and SCHWARZ), with the possible exception of the last named, has made any attempt to repeat it, using the GURWITSCH technique, or even to acquaint himself thoroughly with the theory he is attempting to combat. With this in mind, and considering the rapidly increasing mass of evidence tending to show that all "light," from the shorter radium rays to the short wave radio rays, has very important and as yet little understood effects on living tissues (and in many cases sources in living tissues), it seems to me that we should hesitate to say as yet that "mitogenic rays" are therefore due to join company with phlogiston, abiogenesis, 'n-rays,' and other discarded theories."—P. R. WHITE.⁵

Formaldehyde in photosynthesis.—Ever since it was proposed by VON BAEYER, over 60 years ago, the theory that formaldehyde is an intermediate product in photosynthesis has intrigued the fancy of plant physiologists; but all attempts to demonstrate that it is actually produced during that process have hitherto been either negative in result or open to invalidating criticism. Recently, however, KLEIN and WERNER⁶ have published results of researches which seem to prove definitely that formaldehyde is produced during photosynthesis, and only then. By use of NEUBERG's dimedon method with aquatic plants, it was possible to make determinations on living tissues, and formaldehyde was not only tied up *in vivo* as the stable aldomedon, but also diffused out into the environment almost quantitatively. Very careful account seems to have been taken of the possible sources of error, such as photolysis of sugars, chlorophyll, or organic acids, and assurance is given that no formaldehyde was detectable from these sources with the technique used. Especially convincing is the evidence obtained by use of WARBURG's⁷ technique for inhibiting photosynthesis by anaesthetics which are preferentially adsorbed. When small amounts of HCN or phenylurethane were added, production of formaldehyde ceased at once with cessation of O₂ output, whereas the continued formation of acetaldehyde showed respiration to be unaffected. And when the anaesthetic was removed and O₂ bubbles given off again, formaldehyde was formed once more. Likewise, when any single factor, such as light, CO₂, or temperature, was limiting, no formaldehyde was produced. It is unfortunate that pH measurements were not made, for no assurance is given that control experiments were run at the same alkalinity as the originals reach during photosynthesis. Like-

⁵ See also the review by GICKLHORN (Prague) in *Protoplasma* V. 4:625-628, 1929, of a book: REITER, T., and GABÓR, D., *Zellteilung und Strahlung*, pp. 183, figs. 212. Berlin: J. SPRINGER. 1928, defending on the basis of more than three years' careful experimentation, at the same time that it corrects in many places the work of GURWITSCH and his school, which appeared after the preparation of this note for publication.

⁶ KLEIN, G., and WERNER, O., Formaldehyd als Zwischenprodukt bei der Kohlensäure-assimilation. *Biochem. Zeitschr.* 168:361-381. 1926.

⁷ Review in *Bot. GAZ.* 77:345-346. 1924.

wise one wonders why illumination was always continued for at least 6 hours, when after 2 hours there is marked inhibition exercised by the reagent, or why 12 hours could yield more than 6 hours. But these criticisms do not at all invalidate the essential conclusions established.

More recently KLEIN and SVOLBA⁸ have extended the work to some of the chemosynthetic bacteria, and have demonstrated that at least nitrite and thiosulphate bacteria produce formaldehyde as an intermediate step in their carbohydrate synthesis. They also found acetaldehyde formed during the respiration of these bacteria, as has previously been shown repeatedly for green plants.

In connection with the formation of formaldehyde during carbohydrate synthesis, the work of SABALITSCHKA is of interest. For many years he has studied the ability of plants to form starch and sugar from formaldehyde in the dark, but has left some loopholes for criticism each time. His last publication,⁹ however, seems to offer valid proof that *Elodea* is able to polymerize formaldehyde to sugar and starch, in the absence of CO₂, in either light or dark, and more in dark than in light. The tissue was analyzed before and after the experiment for total hydrolyzable polysaccharides, and the test tissue found not only to contain more than the controls, but to have gained 4-12 per cent over the originals. Other workers,¹⁰ following up this lead, have concluded that evidence from triturated leaf extract shows this polymerization to be due to enzyme action.—H. S. WOLFE.

Rate of photosynthesis.—An attempt to determine the rate of photosynthesis under approximately natural shady conditions has been made by MAXIMOW,¹¹ who measured the amount of CO₂ abstracted from the air by an attached leaf at successive intervals of time. The leaf was introduced into a flat glass vessel from which a rapid air current could be drawn, the air passing over the leaf so rapidly as to prevent any backward diffusion of CO₂. The size of the vessel (40 cc.), and rate of air flow (700 cc. per minute) through it, changed the air about the leaf nearly 18 times per minute. The air flowing by the leaf during 4-8 minute periods was analyzed for CO₂, and equal volumes of air were analyzed for controls. The amount of photosynthesis was calculated in terms of mg. CO₂ used during the brief period.

⁸ KLEIN, G., and SVOLBA, F., Zwischenprodukte bei Assimilation und Atmung autotroper Bakterien. *Zeitschr. Bot.* 19:65-100. 1927.

⁹ SABALITSCHKA, T., and WEIDLING, H., Über die Ernährung von Pflanzen mit Aldehyden. VI. Polymerisation des Formaldehyds durch *Elodea canadensis* zu höheren Kohlehydrate. *Biochem. Zeitschr.* 172:45-57. 1926.

¹⁰ BODNÁR, J., ROTH, L. E., and BERNAUER, C., I. Über die experimentellen Beweisen der Formaldehydassimilationshypothese. II. Die enzymatische Kondensation des Formaldehyds zu Zucker. *Biochem. Zeitschr.* 190:304-325. 1928.

¹¹ MAXIMOW, N. A., and KRASNOSELSKY-MAXIMOW, T. A., Schwankungen im Verlauf der Photosynthese. *Ber. Deutsch. Bot. Ges.* 46:383-391. 1928.

The results showed very sudden and unexpected irregularities in the rate of synthesis, which MAXIMOW believes are natural variations in the speed of the reaction. A few of the results are given here. A buckwheat leaf in six successive 8-minute intervals used respectively 0.680, 0.016, 0.576, 1.032, 0.048, and 0.096 mg. of CO₂. The measurements for barley during eight 4-minute intervals were 0.792, 1.112, 0.964, 1.228, 1.114, 0.774, 0.912, and 1.114 mg., showing within about 40 minutes three rises and two decreases in rate. Similar results were obtained with millet and soy beans. Control determinations of the air itself did not vary over 3-5 per cent, while the leaves showed variations of 25-100 per cent or more.

Two possible causes are suggested for this periodic rise and fall of the rate. Over-accumulation of the synthate might depress the rate, and removal by diffusion might increase it. But more likely the periodicity is caused by periodic changes in the stomatal openings. BLAGOVESTSCHENSKI¹² and AGAMOV¹³ have found that transpiration proceeds in the same fitful manner, and it is logical to assume that stomatal movement would affect the ingress of CO₂ in much the same way that it affects the egress of water vapor.—C. A. SHULL.

Fifth International Botanical Congress, Cambridge, England, 1930

NOTICE CONCERNING MOTIONS ON SUBJECT OF NOMENCLATURE

Motions on the subject of nomenclature for consideration by the Congress should be in the hand of the Rapporteur général, Dr. JOHN BRIQUET, before *September 30, 1929*.

Motions must be presented in the form of additional articles (or amendments) to the Rules of 1905-1910, drawn up in the form adopted in the *International Code*, and must be drafted as briefly as possible in Latin, English, French, German, or Italian. At least one hundred printed copies must be presented.

According to the decisions of the Brussels Congress, 1910, only motions relating to new points which were not settled in 1905 and 1910 can be presented. Motions which do not answer to these conditions shall only be discussed if the Cambridge Congress of 1930 decides to take them into consideration.

For further information about the programme of work for nomenclature, apply to the Rapporteur général, Dr. JOHN BRIQUET, Conservatoire botanique, Geneva, Switzerland.—F. T. BROOKS, Secretary.

¹² BLAGOVESTSCHENSKI, A. W., The botanical station of the Central Asian University in summer. Bull. Univ. Asie Central, Taschkent. Lief. 7:8-14. 1924.

¹³ AGAMOV, SARIBEK, Über die cuticularer Transpiration. Bull. Jard. Bot. Lenigrad 26:576-594. 1927.

THE
BOTANICAL GAZETTE

May 1929

CYTOTOLOGICAL CONDITIONS AND EVIDENCES FOR
HYBRIDITY IN NORTH AMERICAN
WILD ROSES¹

EILEEN WHITEHEAD ERLANSON

(WITH PLATES XVI-XIX AND FOUR FIGURES)

Introduction

Since the appearance of the outstanding papers of TÄCKHOLM (58, 59) on the cytology of *Rosa*, students of this perplexing genus have attempted to evolve satisfactory systems of classification of the multitudinous forms based on the varying chromosome numbers present (BLACKBURN AND HARRISON 4, HURST 31).

In previous studies on the cytology of our North American roses (TÄCKHOLM 59, PENLAND 46), the material has been obtained from the permanent collections in the various botanical gardens of Europe and the United States, the original stations for the material in the field being often unknown. It is not improbable that inter-specific crosses take place between the roses growing together in a large wild rose collection, and since the botanical gardens customarily supply one another with seeds taken from their growing plants, it is possible that after one or two generations of such dispersal some of the "wild species" in a garden may be forms entirely unknown in the field. In the autumn of 1922, the Botanical Garden of the University of Michigan began to build up a collection of North

¹ Papers from the Department of Botany of the University of Michigan, no. 299.

American wild roses from known stations in this continent; this was done with the kind cooperation of many collectors, who responded generously to our appeals and whose efforts make these studies possible. I am particularly indebted to several members of the United States Forest Service, and to Mr. CLYDE LEAVITT of the Board of Railway Commissioners for Canada. Mr. C. C. DEAM and Mr. C. C. EPLING have been most generous in sending interesting and critical forms during the past five years. This collection is intended to aid in the systematic study of *Rosa*, the American forms of which are, many of them, just as polymorphic as are the European types whose chromosome complexes reveal their hybrid nature.

Other workers have reported races within some species of *Rosa* possessing different chromosome numbers based on multiples of seven. TÄCKHOLM, for example, found *Rosa acicularis* Lindl. from Russia to be octoploid, but his Swedish material comprised both hexaploid and octoploid races. PENLAND's North European material was found to be hexaploid, as also Canadian material studied by HARRISON and BLACKBURN (27). TÄCKHOLM had some tetraploid specimens which ALMQUIST identified as *R. blanda* Ait.; PENLAND found *R. blanda* to be diploid. All the unquestionable forms of *R. cinnamomea* L. examined by TÄCKHOLM were diploid, whereas PENLAND (46) and HURST (32) place this species among the tetraploids. Although this is not an American species, it has escaped and has become established in several localities on this continent. The conclusion to be drawn from these findings is either that the chromosome number cannot be taken as a criterion for classifying *Rosa* forms, or else that the material has been incorrectly determined for the cytologists or by them.

In the present investigation many species have been examined cytologically from several parts of their natural ranges. A search was made for wild hybrid forms showing unpaired chromosomes, examples of which have not before been reported in American material. Artificial crosses have been made successfully between forms which differ in chromosome number. Unfortunately *Rosa* is a very unsatisfactory subject for genetical studies, both because of the difficulty in germinating the bony achenes, and because, except in a few Asiatic species, it takes three years for plants to reach maturity.

Material and methods

All the material for cytological study was taken from plants in the Botanical Garden of the University of Michigan, except material from a dozen early-flowering individuals which was kindly fixed by Dr. L. E. WEHMEYER in 1926 from plants in the Arnold Arboretum. The rose collection at Michigan was started at the end of 1922; few plants produced an abundance of flowers until 1926. Fixation was usually carried out in the afternoon, and meiosis was found to be just about as frequent on dull as on sunny days. Following the advice of Professor J. W. H. HARRISON and of Dr. C. C. HURST, the buds were fixed in Carnoy's solution. This fixing fluid has the advantage of rapid penetration, which is important in *Rosa* because the buds are fixed after merely slicing off the apex and base. Carnoy's solution causes a good deal of shrinkage, however, and in the summer of 1927 acetic-alcohol without chloroform was used, at the suggestion of Professor W. P. THOMPSON, who found that this gave a better result in his wheat material than Carnoy's solution. The acetic-alcohol swells the chromatin somewhat, facilitating observations. THOMPSON's method (60) of examining the fixed material after it has stood in 85 per cent alcohol, staining in bulk with acetocarmine, was tried. Although this method does not permit counts to be made, it enables buds in the reduction stage to be picked out before imbedding, thus saving unnecessary cutting.

Flemming's strong mixture, Zenker's solution, and Allen's modification of Bouin's fluid have also been tried. They all give less shrinkage than Carnoy's solution, but the penetration is poor. After these fixing fluids Haidenhain's iron-alum haematoxylin gives better results than after Carnoy's solution, when it sometimes fails to present good contrasts. The modification of Gram's stain as described by CLAUSEN (9) has been found very satisfactory, as has also plain gentian violet, particularly in the case of hybrid forms the protoplasts of which become much distorted by fixation.

For convenience prior to imbedding acetic-alcohol as a killing fluid is recommended, followed by staining with gentian violet; for detailed cytological studies in *Rosa*, strong Flemming or Allen's Bouin solution should be used, followed by iron-alum haematoxylin.

The fixed material was imbedded in paraffin. Sections of diploid

and triploid forms were cut 10-12 μ in thickness, those of tetraploids and higher polyploids at 12-14 μ , thus allowing some complete cells to appear on the sections and facilitating the counting of chromosome numbers.

Polyploid series

All North American wild roses heretofore examined cytologically fall into three groups: (1) the numerous diploid forms with fourteen somatic chromosomes and seven pairs at diakinesis; (2) the tetraploid group comprising *R. virginiana*, *R. carolina*,² *R. arkansana* and their allies, which have twenty-eight somatic chromosomes and fourteen pairs at diakinesis; (3) the *acicularis-nutkana* group which is hexaploid with forty-two somatic chromosomes and seven pairs at diakinesis. I have discovered an octoploid rose and also a few triploid and aneuploid forms on this continent.

RELATIVE CELL SIZES IN ROSA

TÄCKHOLM noticed that the size of microsporocytes at the stage of diakinesis is greater the larger the number of chromosomes, although he did not publish any measurements. The size of individual chromosomes appeared to him to be the same in all the forms of *Rosa*. Table I gives the diameter of microsporocytes measured at diakinesis. Four species or varieties from each group were selected from among diploid, tetraploid, and hexaploid species. These few measurements bring out the fact that diploid types have the smallest cells and hexaploids the largest, although there is considerable overlapping between the cell sizes at diakinesis in tetraploids and hexaploids.

Chromosome numbers

All the forms examined cytologically during the present study are included in the following list (table II). They are arranged according to their chromosome numbers, related types being grouped together in each class. The first column gives the species, accession number, and name of the original collector with date of collection.

² Cytologists should note the nomenclatorial confusion regarding *R. carolina* L. 1753, which is the correct name for *R. humilis* Marsh.; the name has unfortunately appeared in cytological literature as a synonym for *R. palustris* Marsh., the diploid species of the swamps of eastern North America (see RYDBERG 49, and FERNALD 22).

The second column gives the source of the material, usually the field collector's data. The third column contains the number of bivalent chromosomes at first reduction division; in the case of hybrid forms the number of unpaired, univalent, chromosomes present at diakinesis is placed after the number of bivalent chromosomes. The last column gives the number of chromosomes observed in somatic pro-phases. In the first column and immediately following the species

TABLE I
DIAMETER OF MICROSPOROCYTES AT DIAKINESIS

| SPECIES AND ACCESSION NUMBER* | TYPE | KILLING SOLUTION | LARGEST DIAMETER OF CELLS IN μ |
|---|------------|------------------|------------------------------------|
| Rosa blanda 3505 | Diploid | Carnoy's | 6.4 |
| R. pisocarpa 4634 | " | Carnoy's | 7.2 |
| R. palustris 4412 | " | Allen's Bouin | 7.2 |
| R. michiganensis 5891 | " | Carnoy's | 7.2 |
| R. brachycarpa 5301 | Tetraploid | Allen's Bouin | 9.6 |
| R. myriantha 3537 | " | Carnoy's | 9.6 |
| R. obovata 2751 | " | Allen's Bouin | 8.0 |
| R. carolina 5808 | " | Carnoy's | 8.0 |
| R. engelmanni E5 | Hexaploid | Carnoy's | 8.8 |
| R. acicularis var. lacorum 6007 | " | Carnoy's | 9.6 |
| R. acicularis var. rotunda 3511 | " | Strong Flemming | 11.2 |
| R. acicularis E3 (European) | " | Carnoy's | 9.6 |

* Wherever "accession number" is referred to, the reference is to the serial accession numbers of the Botanical Garden of the University of Michigan. "Arn. Arb." numbers are those of the Arnold Arboretum. Nos. E 1, E 2, etc., are my cytological collection numbers for material fixed at the Arnold Arboretum.

name there are small letters which refer to the various kinds of meiotic irregularities observed. The key to these notations is placed at the head of the list.

Extrusion of chromatin has been observed in many cytological preparations of the sporocytes of plant genera. Chromatin or chromosomes are sometimes extruded into the cytoplasm, as found by TISCHLER (61), YASUI (67), LONGLEY (41), HURST (31); or chromatin may pass through the wall into an adjacent sporocyte, as shown by DIGBY (16) in *Galtonia candicans*, by GATES (24) in *Oenothera gigas*, and by WEST and LECHMER (64) in *Lilium candidum*. This has been observed frequently in my material, especially in the prophase stages of meiosis (figs. 6, 15, 51), and is indicated by letter *b* in the first column. The significance of the phenomenon is

not understood, but it is regarded by several cytologists (TISCHLER 62, SAKAMURA 50) as being an indication of degeneration. Recently HURST (31) has used the occurrence of chromosome extrusion in *Rosa* as an argument in support of his theory of the origin of rose types with lower chromosome numbers from the higher polyploids.

In some roses it has been observed that occasionally a microsporocyte will give rise to only two spores (diad) instead of the normal four (tetrad) after meiosis. This has been indicated by *h* in table II.^{2a}

Cytological results

DIPLOID SPECIES

Rosa bracteata Wendl.—This rose, belonging to the small Asiatic section Bracteatae, was not obtained by TÄCKHOLM for his extensive studies. It has become thoroughly naturalized in southeastern United States, where it is common. A plant collected in northwestern Mississippi and grown in the greenhouse proved to be diploid, with seven pairs at diakinesis (fig. 1).

Rosa setigera Michx.—The single American representative of the section Synstylae is very constant in its morphological characteristics, varying chiefly in the amount of pubescence on the foliage. A specimen of the pubescent var. *tomentosa* T. & G. (*R. rubifolia* R. Br.) from Ohio confirmed TÄCKHOLM's observation, showing seven pairs at diakinesis.

Rosa gymnocarpa Nutt.—This species, reported by TÄCKHOLM to have fourteen somatic chromosomes, was found to have seven pairs at the first meiotic division (fig. 2). Material has not yet been obtained from any other member of the section Gymnocarpace.

Rosa palustris Marsh.—The tall swamp rose of eastern and southeastern North America is diploid, as has already been shown by others. It is the largest rose in Michigan, excepting *R. setigera*, and

^{2a} LIST OF CHROMOSOME NUMBERS.—*Explanation of notations:* (a) affinity between members of some pairs at first meiotic reduction weak, resulting in some univalent chromosomes at first anaphase (fig. 20); (b) much extrusion present in material; (c) two ovules observed in one ovary; (d) lagging of some chromosomes at meiotic anaphases (figs. 54, 59); (e) polypory observed at tetrad stage of microsporogenesis (fig. 48); (f) meiosis not synchronous in sporocytes of one microsporangium; (g) rings of four chromosomes observed (figs. 39, 46); (h) diad groups found among tetrads of pollen (fig. 32).

TABLE II

| SPECIES, ACCESSION NO., AND ORIGINAL COLLECTOR | ORIGIN | HAPLOID NO. | SOMATIC NO. |
|---|--|-------------|---------------|
| | | | DIPLOID TYPES |
| <i>R. bracteata</i> Wendl. 3676, L. E. Wehmeyer, 1923..... | Miller, De Soto Co., Miss. | 7 | |
| <i>R. setigera</i> Michx. 3585, unknown. | Ohio | 7 | |
| <i>R. gymnocarpa</i> Nutt. 4006, C. C. Epling, 1923..... | Priest River, Idaho | 7 | |
| <i>Cinnamomeae</i> | | | |
| <i>R. cinnamomea</i> L. (b) 6082, Moses Gomberg, 1924..... | Keene Valley, Essex Co., N.Y. | 7 | |
| <i>R. pisocarpa</i> A. Gray 4634, R. M. Reid, 1923..... | Castella, Shasta Co., Calif. | 7 | |
| <i>R. salicetorum</i> Rydb. 3530, Ehlers and Erlanson, 1923..... | Copeland, Boundary Co., Idaho | 7 | |
| <i>R. puberulenta</i> Rydb. 5594, E. G. Wiesehuegel, 1924..... | Wyo. Natl. For., Sublette Co., Wyo. | 7 | |
| <i>R. macounii</i> Greene 3820, S. Dowding, 1923..... | Edmonton, Alberta | 7 | 14 |
| <i>R. woodsii</i> Lindl. 3208 (plant D), F. B. Cotner, 1923..... | Bozeman, Gallatin Co., Mont. | 7 | 14 |
| <i>R. woodsii</i> Lindl. (a) (e) 4477 (plant N4) Edine Binney, 1923. | Fort Pierre, Stanley Co., S.D. | 7 | 14 |
| <i>R. woodsii</i> Lindl. (b) 3255, A. O. Garrett, 1923..... | Salt Lake Co., Utah | 7 | |
| <i>R. woodsii</i> Lindl. 8152, C. O. Erlanson, 1925..... | So. of Canyon City, Fremont Co., Colo. | 7 | 14 |
| <i>R. pyrifera</i> Rydb. (e) (f) 6610 (2 individuals), A. O. Garrett, 1925 | Salt Lake Co., Utah | 7, 8 | 14, 16 |
| <i>R. ultramontana</i> Wats. 5293, W. H. Ransome, 1924..... | Spokane Co., Wash. | 7 | |
| <i>R. fendleri</i> Crépin (a) 3298 (plant A), F. B. Cotner, 1923..... | Bozeman, Gallatin Co., Mont. | 7 | |
| <i>R. fendleri</i> Crépin 5679, E. L. Perry..... | Santa Fé Natl. For., Sandoval Co., N.M. | 7 | |
| <i>R. hypoleuca</i> W. & S. 8154, C. O. Erlanson, 1925..... | Arkansas River, Fremont Co., Colo. (alt. 5500 ft.) | 7 | 14 |
| <i>R. granulifera</i> Rydb. (b) 5925, W. A. Archer, 1924..... | Black Creek, Sierra Co., N.M. | 7 | |
| <i>R. subblanda</i> Rydb. 3608, L. E. Wehmeyer, 1923..... | Roanoke, Huntington Co., Ind. | 7 | |
| <i>R. blanda</i> Ait. (d) (e) 4071, H. H. Bartlett, 1923..... | St. Ignace, Mackinac Co., Mich. | 7 | |
| <i>R. blanda</i> Ait. 3505, E. W. Erlanson, 1923..... | Bliss Woods, Kane Co., Ill. | 7 | |
| <i>R. blanda</i> Ait. 3942, H. H. Bartlett, 1923..... | Lakeland, Livingston Co., Mich. | 7 | |
| <i>R. blanda</i> var. <i>hispida</i> (d) 5771 (plant B), C. G. Harrold, 1924..... | Gimli, Lake Winnipeg, Manitoba | 7 | |

TABLE II—Continued

| SPECIES, ACCESSION NO., AND ORIGINAL COLLECTOR | ORIGIN | HAPLOID NO. | SOMATIC NO. |
|--|---------------------------------------|-------------------------------|-------------|
| R. blanda var. hispida (a) (b) (d) 5773, C. G. Harrold, 1924..... | Gimli, Lake Winnipeg, Manitoba | 7 | |
| R. blanda var. hispida (d) 2681, E. S. Reynolds, 1922..... | Agric. College, Cass Co., N.D. | 7 | |
| R. blanda var. hispida 2693, Helen Stegenger, 1922..... | University, Grand Forks Co., N.D. | 7 | |
| R. blanda var. hispida 4167, A. H. W. Povah, 1923..... | Beach, Cook Co., Ill. | 7 | |
| R. blanda var. hermanni 2686, F. J. Hermann, 1922..... | Laurium, Houghton Co., Mich. | | 14 |
| R. blanda var. glandulosa (a) (b) (d) (e) 3753, J. H. Ehlers, 1923..... | Prentis Bay, Mackinac Co., Mich. | | |
| Parent plant B..... | | 7 | 14 |
| 2/N38..... | | 7 | |
| 5/N3..... | | 7 | |
| 7/N25..... | | 7 | |
| 7/N36..... | | 7 | |
| Seedling plant 7/N37..... | | 8 | 16 |
| 7/N57..... | | | 14 |
| 7/N62..... | | | 14 |
| 9/N1..... | | 7, 8 | 15 |
| 9/N9..... | | 7 | |
| R. michiganensis (b) (c) (d) (e) 5890, E. W. Erlanson, 1924..... | Douglas Lake, Cheboygan Co., Mich. | 7 | |
| R. acicularioides (a) (d) (e) 8261, H. H. Bartlett, 1925..... | Ogdensburg, St. Lawrence Co., N.Y. | 7 | 14 |
| R. schuetteana (d) (e) 5891, C. and E. Erlanson, 1924..... | Douglas Lake, Cheboygan Co., Mich. | 7 | |
| Caroliniae | | | |
| R. palustris Marsh. 4412, E. E. Gunther, 1923..... | Spider Lake, Grand Travers Co., Mich. | 7 | |
| R. palustris var. inermis (b) (d) (e) 5714, H. H. Bartlett, 1924..... | Quanicassee, Saginaw Bay, Mich. | 7 | |
| R. foliolosa alba (d) (e) 9525, Ralph Shreve, 1926..... | Wetumka, Hughes Co., Okla. | 7 | |
| R. foliolosa alba (d) 9526, Ralph Shreve, 1926..... | Montagu Co., Texas | 7 | |
| R. subserulata (a) (d) 9528, Ralph Shreve, 1926..... | W. Fork, White River, Okla. | 7 | |
| R. rugosa (hyb.) \times R. tetonkaha (b) (d) 5328, N. E. Hansen, 1925..... | Hort., Brookings, S.D. | 7 | |
| TRIPLOID TYPES | | | |
| (?) R. virginiana \times R. palustris (a) (b) (d) (e) (f) (g) 2872, E. C. Robbins, 1922..... | Highlands, Macon Co., N.C. | 7 ₁ 7 ₁ | 21 |
| (?) R. blanda \times R. carolina (a) (b) (d) (e) (f) (g) 2049, W. E. Manning. | Esty's Glen, Ithaca, N.Y. | 7 ₁ 7 ₁ | 21 |

TABLE II—Continued

| SPECIES, ACCESSION NO., AND ORIGINAL COLLECTOR | ORIGIN | HAP-LOID NO. | SO- |
|--|---------------------------------------|--------------|--------|
| | | | MAT-IC |
| TETRAPLOID TYPES | | | |
| <i>Pacific Coast Cinnamomeae</i> | | | |
| <i>R. californica</i> (d) (e) (g) 5849, W. A. Archer, 1924. | Toulomne River, Modesto, Calif. | 14 | |
| <i>R. alderonii</i> (b) (d) (g) 5367, C. C. Epling, 1925. | Griffith Park, Los Angeles, Calif. | 14 | |
| <i>R. brachycarpa</i> (a) (d) (g) 5301, H. Baer, 1924. | Temescal Canyon, Elsinore, Calif. | 14 | |
| <i>R. myriantha</i> (a) (d) (g) 3537, Ellen Bach, 1923. | Nevada City, Nevada Co., Calif. | 14 | 28 |
| <i>Caroliniae</i> | | | |
| <i>R. virginiana</i> (d) (g) 2790, M. Bilon, 1922. | Madison, New Haven Co., Conn. | 14 | |
| <i>R. virginiana</i> (d) (e) 6071, P. F. Weatherill, 1924. | Orr's Island, Cumberland Co., Me. | 14 | |
| <i>R. virginiana</i> (b) (d) (g) 3236 (plant C) Hort. | Naperville Nurs., Naperville, Ill. | 14 | |
| <i>R. obovata</i> (e) (g) (h) 2651, E. W. Erlanson, 1922. | Ann Arbor, Washtenaw Co., Mich. | 14 | |
| <i>R. deamii</i> (d) (e) (g) 3779, C. C. Deam, 1923. | Goldsmith, Tipton Co., Ind. | 14 | |
| <i>R. lyoni</i> (e) 2883, C. C. Deam, 1923 | St. For. Res., Clark Co., Ind. | 14 | |
| <i>R. lyoni</i> (e) 3168, L. F. Brumfield, 1923. | A. & M. College, Oktibbeha Co., Miss. | 14 | |
| <i>R. lyoni</i> (b) (e) (g) 3203, J. T. Buchholz, 1923. | Fayetteville, Washington Co., Ark. | 14 | |
| <i>R. carolina</i> L. (d) (g) 5808, H. H. Bartlett, 1924. | Camp Custer, Kalamazoo Co., Mich. | 14 | |
| <i>R. carolina</i> L. (g) 5291, David Boot, 1924. | Iowa City, Johnson Co., Iowa | 14 | |
| <i>R. carolina</i> var. <i>litoralis</i> (e) 2654, A. H. W. Povah, 1922. | Evanston, Cook Co., Ill. | 14 | |
| <i>R. serrulata</i> Raf. (d) (e) (g) 5566 (plant A), F. K. Sparrow, 1923. | Montgomery Co., Md. | 14 | |
| <i>R. serrulata</i> Raf. (e) 3938, Bartlett & F. C. Blanchard, 1923. | Lakeland, Livingston Co., Mich. | | 28 |
| <i>R. subserrulata</i> (a) (d) (e) 6072, W. A. Archer, 1924. | Rogers, Benton Co., Ark. | 14 | |
| <i>R. petiolarata</i> (d) (e) 3669, L. E. Wehmeyer, 1923. | Boss, Dent Co., Mo. | 14 | |
| <i>R. rudiuscula</i> (a) (d) (e) (f) (g) 3501, E. W. Erlanson, 1923. | St. Charles, Kane Co., Ill. | 14 | |
| <i>R. rudiuscula</i> (a) (e) (g) 4001, C. C. Deam, 1923. | Crown Point, Lake Co., Ind. | 14 | 28 |
| <i>R. rudiuscula</i> (e) 4293, B. F. Bush, 1923. | Greenwood, Jackson Co., Mo. | 14 | |
| <i>Cinnamomeae</i> | | | |
| <i>R. bushii</i> Rydb. (d) (e) (g) 3080, B. F. Bush, 1923. | Courtney, Jackson Co., Mo. | 14 | |
| <i>R. alcea</i> (h) 3571, S. Dowding, 1923 | Calgary, Alberta | 14 | |

TABLE II—Continued

| SPECIES, ACCESSION NO., AND ORIGINAL COLLECTOR | ORIGIN | HAP-LOID NO. | SO-MATIC NO. |
|---|--|--------------|--------------|
| <i>R. subglauca</i> (a) (d) (e) 3492, A. H. Brinkman, 1923..... | Craigmyle, Alberta | 14 | 28 |
| <i>R. suffulta</i> (a) (b) (d) (e) (g) 2682, E. S. Reynolds, 1922..... | Agric. Coll., Cass. Co., N.D. | 14 | |
| <i>R. suffulta</i> (d) (e) (g) 2692, H. Stegenger, 1922..... | University, Grand Forks Co., N.D. | 14 | |
| <i>R. suffulta</i> (d) (e) 2694, H. Stegenger, 1922..... | University, Grand Forks Co., N.D. | 14 | |
| <i>R. suffulta</i> (a) (e) (g) 5598, U.S. For Service, 1924..... | Monument, El Paso Co., Colo. | 14 | |
| <i>R. suffulta</i> (a) (d) (e) (g) 5615, W. A. Archer, 1924..... | Larkspur, Douglas Co., Colo. | 14 | |
| <i>R. suffulta</i> (g) 5705, E. V. Anderson, 1924..... | Concord, Dixon Co., Neb. | 14 | |
| <i>R. suffulta</i> (d) 2897b, H. S. Conrad, 1922..... | Grinnell, Poweshiek Co., Iowa | 14 | |
| <i>R. suffulta</i> (d) (e) (g) 3001, F. C. Gates, 1923..... | Riley Co., Kans. | 14 | |
| <i>R. suffulta</i> var. <i>valida</i> (a) (d) (e) (g) 4459, A. and P. Hamilton, 1923..... | Rockport, Atchison Co., Mo. | 14 | |
| <i>R. arkansana</i> 5771 (plant G) C. G. Harrold, 1924..... | Winnipeg, Manitoba | 14 | |
| <i>R. ratonensis</i> (b) (c) (g) 8114, C. O. Erlanson, 1925..... | Colfax Co., Raton Pass, N.M. (alt. 8800 ft.) | 14 | |
| <i>R. relicta</i> (g) 8320, C. & E. Erlanson, 1925..... | Bliss Woods, Kane Co., Ill. | 14 | |
| HEXAPOD TYPES | | | |
| <i>R. nudikana</i> Presl. 4004, C. C. Epling, 1923..... | Priest River, Idaho | 21 | |
| <i>R. macdougali</i> (d) (e) E. 8, Arn. Arb. 12352..... | Idaho | 21 | |
| <i>R. spaldingii</i> 5549, A. A. Griffin, 1924..... | Naches River, Yakima Co., Wash. | 21 | |
| <i>R. spaldingii</i> 5298, A. A. Griffin, 1924..... | Tieton River, Yakima Co., Wash. | 21 | |
| <i>R. spaldingii</i> E. 4, Arn. Arb. 11116 | Pullman, Whitman Co., Wash. | 21 | |
| <i>R. spaldingii</i> E. 7, Arn. Arb. 1268. | Idaho | 21 | |
| <i>R. spaldingii</i> E. 10, Arn. Arb. | Elk Mt., Idaho Co., Idaho | 21 | |
| <i>R. engelmanni</i> 3254 (plant D), C. C. Moore, 1923..... | Missoula, Missoula Co., Mont. | 21 | |
| <i>R. engelmanni</i> , B. B. Kanouse, 1923 | | | |
| 3719 (e)..... | | 21 | |
| 3721/A..... | | 21 | |
| 3722 (e) (b)..... | | 21 | |
| 3723..... | { Medicine Bow Mts., Albany Co., Wyo. | 21 | |

TABLE II—Continued

| SPECIES, ACCESSION NO., AND ORIGINAL COLLECTOR | ORIGIN | HAP-LOID NO. | SO-MATIC NO. |
|---|--|--------------|--------------|
| <i>R. engelmanni</i> (e) E. 5, Arn. Arb. 9052 A. Rehder, 1916..... | Pikes Peak, El Paso Co., Colo. (alt. 9000 ft.) | 21 | |
| <i>R. butleri</i> Rydb. (a) (e) 8271, C. O. Erlanson, 1925..... | Sangre de Cristo Mts., Custer Co., Colo. (alt. 8400 ft.) | 21 | |
| <i>R. underwoodii</i> Rydb. 8270, C. O. Erlanson, 1925..... | San Isabel Nat. For., Custer Co., Colo. (alt. 8400 ft.) | 21 | |
| <i>R. acicularis</i> var. <i>lacorum</i> 6008, E. W. Erlanson, 1924..... | Prentis Bay, Mackinac Co., Mich. | 21 | |
| <i>R. acicularis</i> var. <i>rotunda</i> 3511, C. O. Erlanson, 1923..... | Douglas Lake, Cheboygan Co., Mich. | 21 | |
| <i>R. acicularis</i> var. <i>bourgeauiana</i> (b) (e) (g) 2592, H. H. Bartlett..... | Mackinaw City, Cheboygan Co., Mich. | 21 | |
| <i>R. acicularis</i> var. <i>sayiana</i> , J. H. Ehlers, 1922 (a) (b) (d) (e) (f) 3754 (plant A), 3754 (plant D)..... | Prentis Bay, Mackinac Co., Mich. | 21 | |
| <i>R. acicularis</i> var. <i>sayiana</i> 6007, C. & E. Erlanson, 1924..... | Scotty Bay, Mackinac Co., Mich. | 21 | |
| <i>R. acicularis</i> Lindl. E. 3 Arn. Arb., 780-5..... | Hort, Kew | 21 | |
| <i>R. acicularis</i> var. <i>nipponensis</i> , (d) E. 14, Arn. Arb., 7839..... | Hort. Chénault, France | 7 | |
| OCTOPLOID TYPE | | | |
| <i>R. acicularis</i> var. <i>lacorum</i> 6447, C. H. Morgan, 1924..... | Fairbanks, Alaska | 28 | |

sometimes reaches over 7 feet in height. Morphologically it is the most highly evolved of the *Caroliniae-Cinnamomeae* east of the Rocky Mountains. In northern Michigan and Wisconsin it has early-flowering races. These are also diploid, and were first recognized by SCHUETTE. Some of these seem to be due to hybridization between *R. blanda* Ait. and *R. palustris*, and are taxonomically distinct. Three of them have already been described as *R. michiganensis*, *R. schuetteana*,³ and *R. blanda* var. *hermanni* (ERLANSOHN

³ *Rosa schuetteana* resembles *R. carolina* L. in some respects, and was at first reported as that species (18). Cytologically it exhibits few irregularities, and has less than 10 per cent of sterile pollen. Since the tetraploid *R. carolina* has not been found in Cheboygan County, Michigan, it seems more probable that this species is descended from *R. palustris* \times *R. blanda*, both of which are diploid forms, instead of from *R. carolina* \times *R. palustris*. The flowering time of *R. schuetteana* is intermediate between those of *R. blanda* and *R. palustris*. This is one case in which cytological examination has aided in the classification of a rose form.

19). These plants exhibit a few irregularities in meiosis, chiefly due to lagging chromosomes during the first and second anaphases. There is a certain amount of polyspory, but the proportion of dwarf pollen grains is small, no larger than in the two hypothetical parents.

HURST (31) thinks that there are five primary groups of diploid species in *Rosa*, which he designates as AA, BB, CC, DD, and EE, each with a distinct set of linked factors. In his report before the Genetical Society at Cambridge, England (32), he designated *R. palustris* as tetraploid AADD and *R. blanda* as diploid DD. *R. palustris*, however, is undoubtedly a diploid species and is typically very distinct from *R. blanda*. These two species would be expected to belong to different primary groups.

DIPLOID SPECIES OF CINNAMOMEAE

A semidouble form of *Rosa cinnamomea* found growing wild in New York proved to be diploid. This record is of interest owing to the conflicting chromosome counts which have been obtained for this species. HURST (32) reported that there exist tetraploid varieties of this species which are "quadrivalent in synapsis."

TÄCKHOLM's findings as to the diploid nature of *R. pisocarpa* A. Gray⁴ (fig. 3), *R. woodsii* Lindl. (fig. 4), and the closely related *R. fendleri* Crép. and *R. macounii* Greene have been confirmed in wild material. Other species from the Rocky Mountain region, related to these, which have been found to be diploid, are *R. ultramontana* S. Wats., *R. salicetorum* Rydb.⁵ (fig. 5), *R. pyrifera* Rydb., and *R. puberulenta* Rydb. These are all tall roses and are characteristic of the waterways. So far as I know, the largest wild roses in any region in this country are the diploid forms, the polyploid forms being as a rule low shrubs growing to 4 feet in height, but usually under 3 feet high in nature.

Rosa granulifera Rybd. and *R. hypoleuca* Woot. & Stand., both

⁴ Specimens of *R. pisocarpa* in most herbaria are of two distinct types: (a) true *R. pisocarpa* with straight ascending infrastipular prickles, oval leaflets, and small hips in crowded corymbs; (b) plants with more scattered weak armature or none, more narrowly oval or obovate leaflets with triangular coarser teeth, larger flowers and hips. These latter belong to the *R. macounii* complex.

⁵ This specific name was misspelled in the original publication as *R. salictorum* (RYDBERG 48).

closely related to *R. fendleri*, are diploid as would be expected (figs. 6, 7).

In the group of *R. woodsii*, reduction takes place in the pollen mother cells when the buds are very small, about 2 mm. in diameter by 5 or 6 mm. long. In the following diploid forms the buds are large, about 4.5 mm. in diameter and 8 mm. or so long, when pollen formation takes place.

As already determined by PENLAND (46), *R. blanda* is a regularly diploid species. I have not been able to find any form of this species showing twenty-eight somatic chromosomes, and do not think that TÄCKHOLM's tetraploid specimens belonged to this species. They may have been some of the forms near *R. bushii* Rydb. and *R. rudiscula* Greene. ALMQUIST determined them as *R. acicularis*.

Rosa subblanda Rydb., a glabrous relative of *R. blanda*, is diploid, as are all the forms of *R. blanda* which have been examined (fig. 8). There are numerous bristly types of *R. blanda*, corresponding to *R. blanda* var. *hispida* Farwell, which are found in widely separated parts of the range of the species, and nearly all exhibit some irregularities during meiosis. The most frequent irregularities are the failure of one or two pairs of chromosomes to be included in the first spindle (fig. 9), or the lagging of some pairs at first anaphase. Rarely at the second division a small extra spindle is observed. Some chromosomes may lag at this division also, and become inclosed in subsidiary nuclei giving polyspory. It is not usual to find more than one extra grain from a pollen mother cell, and counts of mature pollen reveal an appreciable and variable proportion of shriveled grains, from 6 to 33 per cent. These bristly varieties of *R. blanda* when collected in the western parts of its range are usually named *R. macounii*. *R. macounii* is probably a composite species, consisting in part of pubescent varieties of *R. woodsii* (most botanists so interpret *R. macounii*), and in part of more robust forms which should be placed in *R. blanda* var. *hispida*.

R. acicularoides belongs in the group of *R. blanda*, and there are cytological indications that it is of hybrid origin. A specimen from New York was found to be diploid, but some of the chromosomes show a weak affinity at meiosis and in some cells may fail to pair at all. Fig. 20 shows a first meta-anaphase in which four of the chromo-

somes have apparently failed to pair, the members of one pair lying at widely different depths on the spindle. The daughter nuclei will each receive seven chromosomes, however, and several sister interkinesis nuclei have been found, each one of which contains seven chromosomes and a nucleolus. Somatic cells show fourteen chromosomes (fig. 21). The plant from which the fixations were made produces abundant fruit, although castrated buds do not develop. If, under HURST's scheme, it contains two similar differential septets of chromosomes, let us say DD, one would expect to get complete pairing between the seven homologous pairs; if on the contrary it contains two different septets, say C and D, it should be sterile under this scheme.

DIPLOID HYBRIDS

R. blanda glandulosa Schuette

A culture of over 200 seedlings grown from seed of four separate plants of *R. blanda* var. *glandulosa* Schuette is growing at the University of Michigan Botanical Garden. The parent plants were collected in Mackinac County, Michigan, by Dr. J. H. EHLERS in 1923 (accession no. 3753), and the seeds of each were planted separately when they were received from the field. The seeds germinated in 6 months and throughout the first season the seedlings were fairly uniform. These plants came to maturity in 1926 and by then showed great variety. The stems now vary from almost unarmed to densely bristly throughout; in color they vary from red-brown to greenish brown. The hips vary from long-pyriform to globose, and may be either erect or pendulous when ripe. Several of the plants have glandular-compound teeth on the leaflets, as in *R. acicularioides* Schuette. Several plants did not flower in 1926, their third year of growth, and a few of these still failed to produce flowers in 1927. About half a dozen plants were badly cut back by the winter of 1925-1926, in spite of the fact that the parent plants came from latitude 46° north. The majority of the seedlings flowered profusely in 1926 and 1927, at the same time as *R. blanda*, and produced a full crop of large hips full of good achenes. *R. blanda* var. *glandulosa* is characterized by having pyriform hips, and has been called *R. acicularis* \times *blanda* by RYDBERG (48). The morphology of the culture

just described would seem to confirm this diagnosis. Table III analyzes the progeny of the four plants for some characteristics often used to distinguish species in *Rosa*. The majority of the plants bear ellipsoid hips, although these may vary from subglobose to ellipsoid on the same plant. As a rule those individuals which have gland-compound teeth do not have them exclusively; those exhibiting any have been placed in the category "gland-compound." During the

TABLE III

VARIATION IN PROGENY OF FIELD-POLLINATED ROSES

Numbers of specimens exhibiting certain characteristics in cultures grown from seeds of four individual plants of *Rosa blanda* var. *glandulosa* Schuette (no. 3753), from Mackinac County, Michigan; seeds taken from parent plants when received from the field in 1923

| CULTURE AND TOTAL NUMBER OF PLANTS | CHARACTERISTICS | | | | | | | | | | |
|---|-----------------|----------------------------------|--------------------|-------------------------------------|----------------|----------------|--------|-----------|-------|--------|--|
| | Semi- hardy | No flowers in 1926 only | | No flowers in 1926 or 1927 | | Leaflet teeth | | Hip shape | | Sepals | |
| | | Simple | Gland- compound | Sub- globose | Ellip- soid | Glan- dular | Smooth | | | | |
| 3753/2 (50 offspring).... | 3 | 11 | 4 | 41 | 9 | 11 | 32 | 29 | 14 | | |
| 3753/5 (34 offspring).... | 0 | 10 | 3 | 32 | 2 | | | | | | |
| 3753/7* (Total progeny 133, data taken on 75)... | 14 in 133 | 15 | 16 | 67 | 8 | 32 | 12 | 32 | 13 | | |
| 3753/9 (16 offspring).... | 0 | 1 | 1 | 7 | 9 | 1 | 10 | 8 | 1 | | |

* The progeny of 3753 plant 7 contained five plants that bore few flowers and no fruit in 1927, two plants that bore only one deformed hip in 1927; plant N54 had glabrate leaflets and several of the hypanthia aborted after anthesis.

first season the seedlings all had glabrous leaflets with glandular-compound teeth, two characteristics which seem to be universal among *Rosa* seedlings, although in some species the leaflets may become eglandular after the first two or three leaves are produced. In the seedling plants of no. 3753, some show glandular-compound teeth only on the leaves at the tips of turions and vigorous lateral shoots, toward the end of the season. In habit and flowering time most of these plants are like *R. blanda*. They also possess the corymbose inflorescence of that species. A few plants show partial sterility, in that only a few hips are produced and these contain only three or four achenes. One plant (9/N5) flowered but produced no fruit in 1927. Only one plant in the whole culture (7/N117) flowered

appreciably before the rest and showed habit and foliage typical of *R. acicularis*.

Thus far one of the parent plants and nine of the seedlings have been examined cytologically and all but two have been found to be diploids with fourteen somatic chromosomes (fig. 10). Pollen counts made on two of the apparently fertile plants showed 17.6 and 25 per cent of dwarf shriveled grains. The fact that the fixed material of culture 3753 exhibits a great deal of shrinkage, distortion, and chromatin extrusion strengthens my belief that it is of hybrid origin. (CLAUSEN [9] found that the distortion caused by fixation was particularly troublesome in his hybrid *Violae*.) At diakinesis there is a marked delay in pairing among some of the chromosomes (figs. 11, 12), some of which may still be unpaired at the time of heterotypic metaphase. The metaphase plates of the second meiotic division have been found showing seven chromosomes in both daughter nuclei. Some polyspory is evident, as would be expected from the condition of the pollen.

Two of the semihardy plants, which produce no flowers and have only a weak vegetative growth, were brought into the greenhouse, and root tips were fixed in Flemming's fluid, half strong. These showed fourteen somatic chromosomes (fig. 13).

Individual with fifteen chromosomes

Only two of the plants so far examined (culture 3753, plants 9/N1 and 7/N37) showed any extra chromosomes. One of these (plant 9/N1), a plant with glandular-compound teeth, has fifteen somatic chromosomes (fig. 14). At diakinesis there may be more than one univalent chromosome; fig. 15 shows three. There is some lagging at both meiotic divisions. The seven bivalent chromosomes and one univalent may lie on the first spindle (fig. 16), or some may not be included in it. Fig. 17 shows three pairs off in the cytoplasm. Interkinesis nuclei show both seven and eight chromosomes; in fifty cells thirty-five contained seven and fifteen eight. Fig. 18 shows two daughter nuclei at second metaphase. The extra chromosome has already divided and may have done so at first anaphase. In fig. 19 both the daughter nuclei show seven chromosomes at second metaphase, and the extra chromosome has apparently been extruded

and lies on the surface of the shrunken protoplast. This plant then probably produces gametes, mostly with seven, but occasionally with eight, chromosomes. It is a vigorous bush over 4 feet high and bore large crops of tapering hips in 1926 and in 1927.

R. blanda \times *acicularis*

The plants of culture 3753 apparently are a later generation, perhaps many times removed, from a cross between the diploid *R. blanda* and the hexaploid *R. acicularis* of northern Michigan. Such a cross would be expected to give seven bivalents and fourteen univalent chromosomes at reduction division of the first hybrid generation. These hybrids would be expected to be highly sterile, only those gametes receiving few or no univalent chromosomes being able to function. It is possible that the elimination of the univalents through lagging on the spindles at meiosis led to the establishment of the largely stable and fertile diploid type resembling the diploid parent. Such reestablishment of constant types in later generations from crosses between individuals with different chromosome numbers has been found to occur in *Triticum* (KIHARA 38), in *Oenothera* (BOEDIJN 6), in *Papaver* (by a back cross to one of the parent types) (YASUI 67), and has been suggested by HEILBORN (29) as the way in which *Draba magellanica* with twenty-four chromosomes originated.

The great variation in the culture and the presence of some plants with glandular leaflets and pendulous pyriform hips (characters absent in *R. blanda*) suggest affinities with *R. acicularis*, and seem to show that in the original cross seven chromosomes from each parent paired, as would be required by HURST's scheme; but according to that scheme the progeny of such a cross would be sterile. There are in this culture, from four wild unguarded plants, forms that would be named *R. blanda*, *R. blanda* var. *glandulosa*, *R. blanda* var. *hispida*, and *R. acicularioides*, as well as sterile forms.

During the summers of 1926 and 1927 the cross *R. blanda* \times *R. acicularis* and its reciprocal were successfully produced several times. The former cross yields as good a crop of achenes as any cross I have made. *R. woodsii* crossed with hexaploid types has also given good fruit. It may be significant that crosses between diploid and poly-

ploid roses are more often successful when the diploid is the female parent. Attempts to germinate these seeds have so far yielded only one seedling, but there is every indication that these crosses occur and are successful in nature.

It seems highly probable that some of the North American diploid roses have arisen in the manner suggested from crosses between some other diploid with a polyploid form. Such an origin would complicate matters, and would be more in accord with the taxonomic confusion proverbial in this genus than an origin of diploids from polyploids, according to HURST's theory, by the simple loss of sets of seven chromosomes. The variability of the diploid descendants of such crosses is likely to be great, and they can be expected to cross with each other and with the parent forms in the field, to continue to segregate and to give fresh combinations.

The first generation offspring of crosses between parents with different chromosome numbers are probably few and highly sterile, hence inconspicuous in the field. The flowering times of our hexaploid, diploid, and tetraploid roses are characteristic and often distinct, and wide crosses are presumably infrequent in nature. However, the possibility of the production of strong fertile descendants from such crosses in two or three generations permits the taxonomist to ascribe to hybridization the many intermediate forms so notorious in *Rosa* throughout the world.

R. acicularis var. *nippensis* Koehne.

It is pertinent to record here that two plants from two different cultures of *R. acicularis* var. *nippensis* growing at the Arnold Arboretum have been examined cytologically. One is diploid with seven pairs at diakinesis, producing good fruit; the other is almost sterile, seldom producing fruit and the fixed material is very badly shrunk, having seven pairs and also some univalent chromosomes at diakinesis. The plants were grown from seed sent by CHÉNAULT from France. Material that TÄCKHOLM obtained from Kew as this variety he found to be tetraploid. The seeds of *R. acicularis* var. *nippensis* were distributed to the botanical gardens of Europe by the Hortus Petropolitanus about 1870 (WILLMOTT 65). It may be that since then the plants have crossed with others having different

chromosome numbers, until finally a fertile diploid form has been obtained in the same manner that the stable diploid seems to have been produced from *R. acicularis* \times *blanda* in Michigan.

R. rugosa hyb. \times *blanda* (?)

The Tetonkaha rose originated by HANSEN (26), supposedly from the foregoing cross, is diploid, as would be expected from a cross between two diploid species. HURST (32) has reported this cross, and according to his scheme it is CC \times DD and should be sterile, unless it became fertile by a somatic duplication, thereby becoming tetraploid CCDD. The Tetonkaha rose produces large hips resembling those of *R. rugosa*, and apparently crosses with other roses, since seed obtained from another plant growing at Washington, D.C. gave large seedlings with the habit of *R. multiflora* or *R. arvensis*. These latter, however, do not set fruit. Apparently crosses between very distinct diploid species may give fertile offspring.

MEIOTIC IRREGULARITIES IN DIPLOIDS

The early history of meiosis in all the species examined has corresponded with that described by BLACKBURN and HARRISON (4) for *R. arvensis*. The chromosomes are arranged end to end, and sometimes pairing is considerably delayed after strepsinema during condensation, so that strings of chromosomes appear (fig. 5). Frequently the members of some pairs become attached by their ends alone, thus forming a ring (fig. 12), these configurations recalling similar ones in *Oenothera*. Frequently two chromosomes in a nucleus will delay pairing until the first spindle is formed. In some cases, although all the chromosomes may come to lie on the equator of the first spindle, the members of one pair may fail to associate with each other (fig. 20). It is noticeable that condensation proceeds irregularly among the chromosomes after fragmentation of the spireme, so that it is not unusual to find the majority of the pairs well condensed, with one or two still long and threadlike. This state of affairs is also true among the tetraploids (fig. 38 A).

Meiosis in North American diploid roses is seldom completely regular as it was found to be in *R. arvensis* by BLACKBURN and HARRISON. Most of the forms studied resemble cytologically the

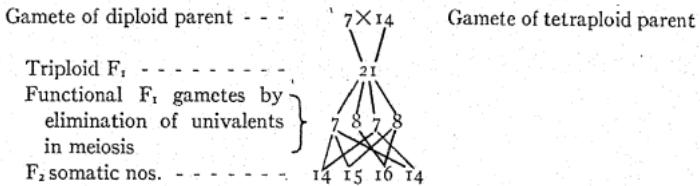
diploid hybrids examined by TÄCKHOLM. Sometimes one or more pairs of chromosomes fail to reach the first spindle (figs. 9, 17), and may lag behind the others at first anaphase. The interkinesis nuclei usually receive seven chromosomes, but there may be more lagging at second anaphase. These irregularities are reflected in the presence of polyspory (fig. 24) and in the pollen counts (table IV), which show a considerable proportion of shriveled grains for species in which PENLAND considers hybridization scarcely to exist.

Extrusion of chromosomes, upon which HURST lays so much stress, is frequent in all stages of meiosis, and is more noticeable in the more sterile plants than in others. In how far this phenomenon is an artifact is not yet known; my material seems to show that in many cases it is probably due to imperfect fixations. Fig. 6 shows two contiguous cells in which extrusion at diakinesis is commencing; the nuclear membrane in both cells has been drawn out nearer to the edge of the protoplast and has been broken through in the same relative position, which suggests a too sudden penetration of the fixing fluid from one side. The number of chromosomes extruded may vary, as found by LONGLEY (41) in triploid *Rubus* species. Occasionally all the chromosomes of many cells in a loculus are found to be extruded, or more rarely all the chromosomes of a cell are extruded into the cytoplasm of a neighboring pollen mother cell (fig. 15). Even according to HURST's theory there does not seem to be further need for extrusion once the plants have become diploid.

TYPES WITH SIXTEEN CHROMOSOMES

A small culture of plants (no. 6610) grown from seed sent by Mr. A. O. GARRETT from Utah, from a plant identified at the United States National Herbarium as *R. pyrifera* Rydb., exhibits a considerable amount of variation. All have glabrous leaflets as in *R. woodsii*. One plant has sepals that are erect on the fruit; another has sepals that are spreading or reflexed, with small lateral pinnae. The fruit of the latter resembles that of *R. californica*, and it also ripens later than that of the former plant. In 1927 very few flowers were produced, and, unfortunately, buds from two plants were fixed together. Cytological examination revealed that one plant is a regular diploid while the other has an extra pair of smaller chromo-

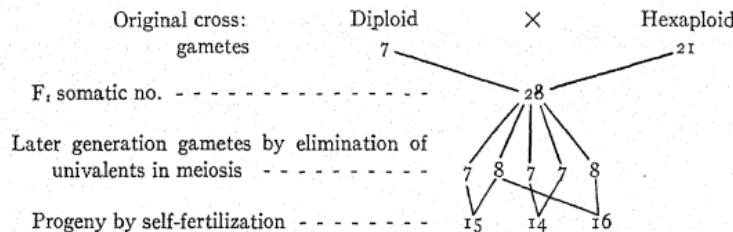
somes, giving eight pairs at diakinesis (figs. 22, 23). The bud with sixteen chromosomes also exhibits a phenomenon pertaining to nuclear disturbance which is absent in the diploid bud; that is, meiosis does not take place synchronously. Scattered in a single anther loculus some tetrads of young pollen grains can be found, as well as sporocytes undergoing reduction division and also some at diakinesis. This condition was noticed in the pollen sacs in the perfect flowers of *Populus tremuloides* (ERLANSON and HERMANN 20); it is said by TÄCKHOLM (59) to characterize hybrid forms of *Rosa*. It is probable that the plants from Utah are descendants from a cross involving a diploid (perhaps *R. woodsii*) and some polyploid form (perhaps a tetraploid). In our culture 3753 a plant with fifteen chromosomes (plant 9/N1) and one with sixteen chromosomes (see later) have been discovered. All these aneuploid forms may have been produced from viable gametes that possessed eight instead of seven chromosomes. A hybrid plant with fifteen chromosomes might produce several gametes with eight chromosomes, and by self-fertilization give rise to offspring with sixteen chromosomes. It would also produce gametes with seven chromosomes and could therefore give progeny with fourteen and fifteen chromosomes, as shown in the following diagram:



It is significant that one of the pairs at diakinesis in the bud of *R. pyrifera* with sixteen chromosomes is smaller than the others. CLAUSEN (9) reports that in *Viola* hybrids in which the unpaired chromosomes divide at both meiotic divisions, the chromosomes that result are smaller than the others in the next generation.

Material fixed from plant no. 7/N37 in culture 3753 of *R. blanda glandulosa* showed no reduction stages in the microsporocytes. In one ovule, however, four young megasporites were found each showing eight chromosomes (fig. 25). The chalazal megasporite in the row is

already shrunken as if about to disintegrate; the two nearest the micropylar region are side by side instead of being in linear order, but one is larger than the other. TÄCKHOLM (59), and earlier STRASBURGER (57), found that in *Rosa* the micropylar megasporangium is the functional one. Somatic counts of this plant show clearly sixteen chromosomes (fig. 26). It is conceivable that the plants of culture 3753, showing as they do fourteen, fifteen, and sixteen chromosomes, all arose from a fertile hybrid ancestor with fifteen chromosomes, suggested diagrammatically as follows:



Aneuploid forms are rare in *Rosa*. TÄCKHOLM only found five among 293 individuals; I found three among 108.

TRIPLOID TYPES

Among over 100 individuals which have been examined cytologically from the collection at Michigan, only two (and a possible third) have been found to be triploid, possessing twenty-one somatic chromosomes.

R. palustris \times *carolina*, no. 2872

Specimens received from North Carolina as *R. lucida* (syn. *R. virginiana*) are vigorous and form a dense thicket. The stems are over 3 feet high, greenish brown, and weakly armed; they bear many flowers in small corymbs and begin to bloom in the second or third week of June at Ann Arbor. After anthesis all the hypanthia usually shrivel and fall off. Very rarely one or two small hips are formed, each containing only one or two achenes. The hypanthium in flower is glabrous; the pedicels bear a few stipitate hispid glands; the achenes are basally attached; and the sepals remain reflexed or spreading on the occasional hips.

A great deal of shrinkage and distortion due to fixation gave

similar effects to those noticed by CLAUSEN in his *Viola* hybrids, and impeded cytological examination. At diakinesis in the microsporocytes seven paired and seven unpaired chromosomes appear (fig. 27), the latter being noticeably small. All these elements become grouped on the equator at the first meiotic metaphase (fig. 28); and one member of each pair passes to each pole. The univalents become scattered along the spindle, and usually pass as whole chromosomes to the poles, being distributed at random. There is a tendency for the univalents to become fragmented at this stage, although this may be due to the fixing fluid. A similar effect was observed by GATES and THOMAS (25) in pollen mother cells of *Oenothera lata*, which is also a highly sterile form due to the presence of an extra unpaired chromosome. Lagging, unpaired chromosomes occasionally divide at the equator in the first anaphase; but only rarely, since, in the few sister interkinesis nuclei observed, in no case did the sum of their chromosomes total more than twenty-one. At late interkinesis and second metaphase varying numbers of chromosomes (usually 8-12) were found. The material does not show many cells in this stage, and only one case was observed in which the daughter nuclei contained either seven or fourteen chromosomes. Fig. 29 shows sister second metaphase plates with nine and twelve chromosomes respectively. Since no chromosomes are visible scattered in the cytoplasm, it is to be concluded that the unpaired seven passed whole to the poles, two going to one and five to the other. The second meiotic division exhibits several irregularities; extra spindles with two or three chromosomes are not infrequent. On the main spindles all the chromosomes usually begin to split and to separate simultaneously, but during anaphase some of the halves lag behind and fail to become included in the major grand-daughter nuclei. Apparently some of the chromosomes may fail to split at second anaphase and instead pass whole to the poles. Fig. 30 shows a second meta-anaphase spindle on which the seven large chromosomes (originally conjugants) have divided or are in the process of dividing; of the seven smaller chromosomes (originally univalents) three are dividing at the equator, one lies partly split at one pole, and the other three seem to be passing entire to the poles, two to one and one to the other. If all the chromosomes reach the poles,

the two resulting microspores will receive twelve chromosomes each, but in an entirely irregular manner. Polyspory is much in evidence. There are a few tetrads of pollen grains but usually from five to ten cells result from one sporocyte (fig. 31). In some microsporangia a few sporocytes fail to undergo meiosis, and can be observed full of fat globules, apparently breaking down among young microspores. Other sporocytes may give rise to two pollen grains only, and these may both be approximately of the same size and both large (fig. 32); rarely one member of a diad is almost twice the size of the other. It has not been possible to observe whether such diads are the result of a microsporocyte failing to develop beyond the first meiotic telophase, or whether at second telophase the chromosomes become included in two nuclei as observed by BLACKBURN and HARRISON (4) in *R. sabini*.

Culture 2872 is thus seen to be a good example of the "*Drosera*-type" of meiosis, and therefore resembles in its cytological behavior four of the five triploids whose meioses were observed by TÄCKHOLM. This type of meiosis is interpreted as being due to the fact that all the members of a smaller gametic chromosomal complement pair with members of a larger gametic complement, in the sporocytes of hybrids from a cross between two parents having different chromosome numbers. It is further characterized by TÄCKHOLM as having all the unpaired chromosomes irregularly distributed as whole chromosomes to the poles at first anaphase. It was originally observed by ROSENBERG (47) in his *Drosera rotundifolia* \times *longifolia* hybrid, and since then in hybrids of *Morus* by OSAWA (45), of *Triticum* (SAX 51, 52), of *Fragaria* (ICHIJIMA 33), and of other plant genera. Complete accounts are given by TÄCKHOLM (59), SHARP (55), and MORGAN (44).

Since these *Rosa* plants came from North Carolina and their cytology reveals them to be offspring from a cross between a diploid ($x=7$) and a tetraploid ($x=14$), it is not difficult to assign putative parents. The only diploid species of the Cinnamomeae-Carolinae in the region is *Rosa palustris* Marsh., the tetraploid species being *R. carolina* L. and its varieties (or closely related and doubtfully distinct species), and possibly *R. virginiana* Mill. The triploid plants form dense thickets like those of *R. palustris*, although the habit is

more slender and weedy. The leaves are narrow and lance-elliptic, with finer teeth than in *R. carolina* and its allies, or than in *R. virginiana*. It flowers with the two latter species, two weeks or more before *R. palustris*. The occurrence of true *R. virginiana* in this region is doubtful, so that it is highly probable that these plants are hybrid *R. palustris* \times *carolina*, the *R. humilis gracilis* of Porter MS according to RYDBERG (49). Our plants have weak, straight, infrastipular pickles or none, and the stems are bristly at the base. The fact that the hypanthia are glabrous is not significant, since the characteristic hispid glands are not infrequently absent from some individuals in both *R. palustris* and *R. carolina*.

Pollen examination revealed very little pollen in a flower bud, and of what was produced not a single grain was observed which was plump and yellow; all the grains were transparent and wrinkled, 60 per cent of them being misshapen and the pollen 100 per cent bad. In examining ovules from these plants no stages of meiosis have been observed, nor any embryo sacs. Some tetrads of megasporangia have been seen, and in some cases all four spores appeared to be disintegrating. In one case the megasporangium nearest the micropyle was still large while the other three were shrunken.

Culture 5590 (?) R. palustris \times *virginiana*.—In a small culture of *R. palustris* grown from seeds sent by Dr. H. M. DENSLAW from New Jersey, six seedlings out of nineteen were noticed to have gland-compound teeth during the first season. In the following year (1926) all had simple teeth. They flowered for the first time in 1927, one plant beginning to bloom 8–10 days before the others. Unfortunately it was not observed until it was almost past flowering, and buds fixed then showed neither meiosis nor mitosis. However, a great deal of shriveled pollen was evident, and a count of mature pollen showed only 3 per cent of good grains. After flowering all the hypanthia shriveled and fell. In one bud 88 stamens were counted, about 100 less than the usual number in *R. palustris*. The plant is low in stature and the leaflets are distinctly ovate with coarse teeth; the other plants in the culture are typical of *R. palustris*. This sterile plant is therefore suspected of being a triploid hybrid, perhaps *R. palustris* \times *virginiana*. This case emphasizes the necessity for growing large cultures of wild roses in order that the sterile and often

weak hybrid forms with interesting chromosome complements may appear, as well as to give an adequate idea of variation. It is often very difficult to obtain cultures because of slow and poor germination.

Culture 2949 (?) R. blanda \times *carolina*.—The second plant which has been demonstrated cytologically to be a triploid with twenty-one somatic chromosomes was sent from Ithaca, N.Y., by Mr. W. E. MANNING. It is a low weak plant about 18 inches high, and produces very little vegetative growth. It is almost unarmed, possessing only a few weak paired infrastipular prickles. The leaflets, usually seven, are glabrous and glaucous, or have a few hairs on the veins beneath. The teeth are fine and ovate, smaller than is usual in *R. blanda* or *R. carolina*. It flowers toward the end of June, at the same time as *R. carolina*, bearing solitary flowers on the old wood but small terminal corymbs on some of the season's turions. Subglobose hips about 1 cm. in diameter are set. These and the pedicels are entirely glabrous. After anthesis the sepals are reflexed or spreading but are persistent. The achenes are basally attached, as is usual in *R. carolina* and occasional in *R. blanda*. The stamens vary from 140 to 155 per flower, about as in *R. carolina*; in fact, the plant strongly resembles some forms of *R. carolina* which are almost unarmed and eglandular.

At diakinesis in the microsporocytes of this triploid plant some cells show seven pairs of conjugating chromosomes, although fewer usually appear (fig. 33), and in a few cells the affinity is so weak that all twenty-one appear as distinct units, although some are grouped together in pairs they are not closely appressed. There is some extrusion of chromosomes at this stage, but so far as observed the number that pass from one cell into the next is variable, from one or two to all the chromosomes.

At early first metaphase all the bivalent and univalent members become grouped somewhat irregularly on the equator, the members of the pairs beginning immediately to disjoin and pass to opposite poles. The univalents split and seven half chromosomes often pass to each pole after the seven whole ones (fig. 34). There does not seem to be any definite grouping of the univalents and bivalents on the first metaphase plate, as is the case in many of the Caninae; in

fact, it is usual for some of the chromosomes to lie at various levels on the spindle, giving an irregular effect. Some of the univalents seem to have difficulty in dividing, so that the chromatic material becomes drawn out along the spindle fiber between the two halves. In some cells a few of the univalents fail to split, passing to one of the poles entire at first anaphase. In one such cell observed, nine chromosomes could be counted at one pole while four univalents were to be found dividing at the equatorial region; in another there were also nine chromosomes at one pole and five univalents were dividing at the equator. The distribution of chromosomes in 100 second metaphase plates was counted, and is shown as follows:

| | | | | | | | | |
|-------------------------|---|---|----|----|----|----|----|----|
| No. of chromosomes..... | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| No. of plates..... | 1 | 4 | 14 | 23 | 15 | 13 | 12 | 18 |

The following distribution was observed in seventeen pairs of sister second metaphase plates. One pair had 14 and 13 chromosomes respectively; one had 14 and 12; two had 14 and 10; one had 14 and 9; one had 14 and 8; one had 14 and 7; one had 13 and 13; three had 13 and 12; two had 13 and 9; one had 12 and 11; one had 12 and 10; one had 11 and 10; and one had 10 and 10. In only five cases out of these seventeen does the sum of the chromosomes in two sister nuclei amount to only twenty-one or less, which might mean that in those cells none of the univalents had divided at first anaphase, providing all the chromosomes reached the poles. If all the univalents divided at first anaphase the daughter nuclei should each receive fourteen chromosomes. The two factors which prevent the sums of the chromosomes of sister nuclei from being twenty-eight are: first, all the univalents do not divide in every cell at first anaphase; second, all the split univalents are not included in the daughter nuclei. Nevertheless 30 per cent of these receive thirteen or fourteen chromosomes (fig. 35), and only in one case were sister plates observed containing seven and fourteen chromosomes respectively.

The second anaphase is usually initiated very regularly, all the chromosomes lying in one plane on the equator and all beginning to split simultaneously, in contrast with the conditions found in 2872. By the time second telophase is reached, however, some of the

chromosomes may lag behind the rest and fail to reach the poles. Many of the microsporocytes give rise to tetrads of microspores, and, although polyspory is frequent there are seldom more than two or three subsidiary spores produced. Examination of the pollen showed more shriveled grains than was expected, only 7.3 per cent of large plump grains being found in over 400 counted.

This plant (2949) resembles cytologically the triploid *R. centifolia major* examined by TÄCKHOLM (as no. 194), and the percentage of good pollen is very little more than that found in *R. centifolia major* by HURST (30). This type of meiosis has been called the "Rosa-type" by TÄCKHOLM, who first found it fully developed in the microsporocytes of the Caninae wherein all the unpaired chromosomes split at first meiotic anaphase. Our triploid from New York approaches very nearly to the *Rosa* type of meiosis, since it appears that nearly all the unpaired chromosomes divide at both meiotic divisions. The triploid from North Carolina, on the other hand, behaves almost as ROSENBERG's *Drosera* hybrids, only rarely any of the unpaired chromosomes dividing at first anaphase.

Possible occurrence of fertile triploids

The outstanding point of interest about the New York triploid type is the fact that it produces a crop of good hips and achenes. As all students of *Rosa* know, the cytological examination of meiosis in the megasporocyte is difficult. Since our plants are small it has been possible to fix only a few buds; flowers have been needed for herbarium vouchers and also for seed. In ovules of accession no. 2949 good normal embryo sacs have been seen, as well as one case of two ovules in one carpel and a case of two embryo sacs in one nucellus. Such abnormalities have been observed before in the genus (STRASBURGER, TÄCKHOLM), and are considered by some to be characteristic of hybrids (JEFFREY 35, 36). No stages of meiosis have yet been discovered in the ovule, and three castrated flowers gave no fruit. In the absence of direct evidence as to chromosomal behavior during megasporogenesis, there are two alternate possibilities to be considered as explanations for the fertility of this plant.

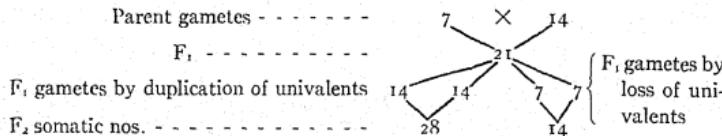
As a first alternative the plant may resemble the Caninae, in which case all the unpaired chromosomes in the megasporocyte pass

as a group to one pole at first meiotic anaphase, and the daughter nucleus receiving fourteen chromosomes gives rise to the functional megaspore. If, as in the Caninae, only those pollen grains containing seven chromosomes are functional, there would be only a small chance for this plant ever to produce zygotes by self-pollination, if one judges from the distribution of chromosomes in second meiotic metaphase nuclei. By the time second telophase is reached some grand-daughter nuclei may receive only seven chromosomes, due to lagging during the second anaphase, as is the case in the Caninae. Should the specimen correspond in this way to the roses of the Caninae, it would thereby give rise to a freely fruiting triploid race of North American roses, either by apomixis or by the fusion of a fourteen-chromosomed egg with a seven-chromosomed sperm nucleus. The extreme rarity of triploids in my material, and the fact that none have been reported by others from this continent, would seem to preclude this possibility, although our plant might be the starting point for such a line. Such triploids, strongly resembling tetraploid types, as does our plant, and setting fruit freely, would be very difficult to recognize as hybrids in the field unless one examined the pollen.

The second alternative is that meiotic behavior in the ovules of this plant corresponds to the process in the microsporocytes, as is the case in other Cinnamomeae. If this were so we could expect an appreciable number of both megasporocytes and microspores to receive fourteen chromosomes, and there would be produced a zygotic tetraploid form with twenty-eight chromosomes from a triploid parent by self-pollination. The plant would then resemble CLAUSEN'S *Viola* hybrid, in which the somatic number in the F_2 generation was increased over that in the F_1 by a double splitting of unpaired chromosomes during meiosis. KARPECHENKO (37) also found that this took place in some *Brassica* \times *Raphanus* hybrids. The increased chromosome number observed by BREMER (8) in a hybrid from a species cross in *Saccharum* may have come about in this way. The possibility that our plant may produce some spores that receive only seven chromosomes is not ruled out.

In the central United States there are many *Rosa* forms which are very puzzling taxonomically. Some of them appear to be inter-

mediate between *R. suffulta* (tetraploid) and *R. blanda* (diploid), for example, *R. relicta* Erlanson. It may be that these actually originated from crosses between diploid *R. blanda* and tetraploid forms, and that the resulting triploid hybrids were fertile as is 2949, giving rise to fertile tetraploids or perhaps to some diploids in the following manner:



According to HURST's theory of differential septets, if the two parents were respectively CC and CCDD, there would be the re-establishment of the tetraploid parent type by the duplication of the unpaired D chromosomes in the *F*₁ hybrid. To a certain extent this is true, since the rose forms in question have many characteristics of other tetraploids, yet they are distinct from *R. suffulta* and exhibit some characteristics of *R. blanda*.

The tetraploid *R. damascena* is known to have given rise to a triploid form in cultivation, and HURST (31) believes this to be due to the loss of a septet by extrusion. Since our fertile triploid strongly resembles *R. carolina*, it might be attributed to a loss of seven chromosomes in one of the parent gametes, but the idea of its possible hybrid origin seems to be more in conformity with known proceedings in other organisms.

In 1927 six field-pollinated hips were collected from 2949, containing 13, 8, 6, 5, and 4 achenes respectively. Out of three buds which were self-pollinated, one contained only two achenes and the other none. Too much emphasis must not be laid on the apparent failure of self-pollination, since it has been found very difficult to obtain seeds in this way from many of the North American *Rosa* species, particularly in *R. blanda*. It may be that bagging the whole inflorescence causes the pollen to become damp and non-functional, or there is the possibility of some self-sterility. FOCKE (23) gives *R. blanda* \times *lucida* as a garden hybrid.

Infrequency of triploid roses

The fact that I have been able to find so few triploid roses is surprising, especially considering the great number of triploid forms LONGLEY (41, 42) found in *Rubus* and *Crataegus*. One reason for this no doubt is the fact that our tetraploid and diploid species of *Rosa* are so often separated both in space and in time of flowering. In the eastern part of the continent *R. blanda* (diploid) flowers before *R. virginiana*, *R. carolina*, and their allies (tetraploids), while *R. palustris* (diploid) comes into flower last of all. In the western part *R. woodsii* and its allies (diploid) flower before *R. suffulta* (tetraploid) and before *R. californica* (tetraploid). Also, the diploid forms are characteristic of waterways and swamps, while the tetraploids are usually found on dry uplands and plains. Further extensive field work will no doubt bring to light other hybrids between the two groups, and crosses already have been successfully made experimentally.

TETRAPLOID TYPES

There are two very distinct groups of tetraploid rose species in North America, widely separated geographically. These are the *R. californica* group on the Pacific Coast, and the large group comprising *R. virginiana*, *R. carolina*, and *R. arkansana* and their allies. The former two are distributed over the eastern half of the continent north to the Great Lakes and the St. Lawrence, and the latter is characteristic of the great plains of the Mississippi drainage system.

R. californica and allied species.—TÄCKHOLM (59) found *R. californica* to be diploid with seven pairs at diakinesis. I have examined one individual of this species and one of each of the closely related species *R. brachycarpa* Rydb. and *R. alderonii* Greene, which are perhaps only varieties of *R. californica*. All three show fourteen pairs of chromosomes at reduction division in the pollen mother cells (figs. 38-40). The allied species *R. myriantha* Carr, obtained from Nevada City, which morphologically is somewhat intermediate between *R. californica* and *R. pisocarpa*, is also tetraploid (fig. 41).

Middle-western and eastern species.—Cytological examination has confirmed TÄCKHOLM's findings that *R. virginiana* Mill., *R. carolina* L. (*R. humilis* Mill.), and *R. suffulta* Greene all have twenty-eight somatic chromosomes and fourteen pairs at reduction division.

This group of roses is extremely variable and difficult taxonomically. In addition to the usual morphological variations, this group shows marked geographical and local races which present great difficulties to descriptive definition. It has long been a moot point whether *R. carolina* is a variety of *R. virginiana* or not because of the intergrading series of forms found between the two in the northeastern United States (BEST 2). In the middle western region there is an equally notable intergrading series of forms between *R. carolina* and its varieties and the *R. arkansana-suffulta* group, giving forms like *R. rudiulus* Greene and *R. conjuncta* Rydb.

Tetraploid and diploid races of R. subserrulata Rydb.—When a specimen from Oklahoma, which agrees morphologically with *R. subserrulata* Rydb., was examined cytologically, it was very unexpectedly found to be diploid, showing seven chromosomes at first and second metaphases with only a slight amount of lagging during meiosis, and an occasional chromosome off in the cytoplasm. Another specimen belonging to this species, from Arkansas, was examined and found to be tetraploid, as would be expected from its close resemblance to *R. carolina*. The tetraploid plant (no. 6072) shows several irregularities during meiosis. At first anaphase from three to five pairs may lag behind the others; fig. 59 shows three lagging pairs as well as two unpaired chromosomes, one of which is beginning to divide. Early first telophases usually show fourteen chromosomes (fig. 61), and in one case two distinct groups of seven chromosomes were found at this stage (fig. 60). Except for this example, which could be due to chance grouping, I have not seen any evidence for chromosomes separating in groups of seven at reduction division as HURST's theory would seem to demand, if the septets, which he regards as linkage groups, are to remain intact. According to HURST's theory the diploid *R. subserrulata* might be regarded as having been produced from a tetraploid form by loss of a diploid set of septets. The origin of both the diploid and tetraploid types of *R. subserrulata* can also be postulated from a fertile triploid producing functional gametes with both seven and fourteen chromosomes, as suggested under the New York specimen 2949.

This case of *R. subserrulata* is one of only two in which have been found different chromosome numbers in two individuals apparently

belonging to the same species. The diploid *R. subserrulata* was received from Mr. RALPH SHREVE late in 1926, and flowered in the cold frame in 1927. At present I do not know whether it produces flowering turions or not. The common widespread glandular form of *R. carolina*, *R. serrulata* Raf. (*R. carolina* var. *glandulosa* Farwell), was found to have twenty-eight somatic chromosomes in a Michigan specimen (fig. 47) and fourteen pairs at diakinesis in a specimen from Maryland (no. 5566, plant A).

Meiosis in megasporocyte.—The diad stage of meiosis was observed in an ovule of a plant from Aurora, Illinois (no. 3501) which I consider to be a form of *R. rudienscula* Greene (fig. 62). One of the cells is in interkinesis while the other is entering second metaphase. Both show fourteen split chromosomes.

MEIOTIC IRREGULARITIES IN TETRAPLOIDS

Early stages of meiosis in the tetraploid forms resemble similar stages in the diploid types. The chromosomes are attached end to end, and each usually becomes paired with one adjoining individual. Condensation of the chromosomes is irregular. Fig. 38 shows a diakinesis nucleus in *R. californica* in which there are twelve pairs, a ring of three and one long, unpaired chromosome. Rings of four chromosomes are often present during diakinesis, but they usually break up into two pairs by the time the first meiotic metaphase is reached, and well condensed groups of four are not as common as are rings (figs. 42, 52). There is most frequently only one ring of four to a nucleus, although two or even three have been observed (fig. 51). Fig. 46 shows a cut cell at early first anaphase in which a ring of four chromosomes has not yet succeeded in condensing and reaching the spindle.

In some nuclei at diakinesis there seems to be a tendency for half of the chromosomes to pair intimately quite early, while the other fourteen lie adjacent to each other in pairs but seem to have a decidedly weaker affinity. This is particularly noticeable in species of the *R. suffulta* complex (figs. 49, 53). Some of the members of this group sometimes possess certain characteristics of *R. blanda*, and are often associated with it in the field; and, as has been suggested, may have arisen as a cross between that species and some

tetraploid. The discovery of the fertile triploid 2949, which could conceivably give rise to a fertile race of tetraploids by self-pollination, suggests a possible origin for such anomalous tetraploid forms. One would expect that chromosomes which were originally halves of a single individual would pair very intimately at diakinesis.

In some cases a few of the chromosomes in a tetraploid microsporocyte fail to pair at all at diakinesis, and, just as among some diploids, they appear on the first metaphase spindle as univalents (fig. 56). During first anaphase there is nearly always some lagging of one or two pairs, but each pole usually receives fourteen (figs. 40, 43). It is also evident that occasionally some of the chromosomes that fail to pair will divide at late first anaphase (figs. 54, 57, 59). This was observed by TÄCKHOLM in his tetraploid specimen no. 132, which was named *R. blanda*, as well as in some of his tetraploid hybrids. Second anaphase may be regular (fig. 50), or there may be some lagging and extra nuclei at second telophase (fig. 58).

Cytologically nearly every tetraploid specimen examined showed meiotic irregularities which differ only in degree from those found in known tetraploid hybrids examined by TÄCKHOLM. A dwarf plant of *R. subglauca* Rydb. from Alberta (no. 3492) shows many such irregularities. At first meiotic metaphase nearly all the chromosomes lie loosely associated in pairs (fig. 55) or some fail to pair. Fig. 56 shows four univalents on the spindle. Some of them split at first anaphase (fig. 57), and there is some lagging so that all do not reach the poles. Fig. 58 shows a sporocyte at second telophase in which one pair of grand-daughter nuclei contain fourteen chromosomes each; the other pair twelve and thirteen respectively. Five extra chromosomes and two split ones lie in various positions about the cell, making a possibility of nine chromosomes not included in the four nuclei. Since this makes a total of sixty-two chromosomes instead of a normal fifty-six, three univalents have divided at both metaphases.

Polyspory is more frequent among tetraploids (fig. 48) than among diploids, and the percentage of bad pollen is larger on the average (table IV). Extrusion is frequent, as in the diploids, and the number of chromosomes thus lost seems to be arbitrary.

POSSIBLE ORIGIN OF TETRAPLOID TYPES

One of the most striking characteristics of meiosis in the tetraploids is the presence of groups and rings of four (instead of two) chromosomes at diakinesis. This is shown in several of the figures. TÄCKHOLM does not seem to have observed it. It is frequent in my material and resembles the configurations found in tetraploid *Datura* plants by BELLING and BLAKESLEE (1), although in no case has a cell been observed that is completely quadrivalent in synapsis. The tetraploid condition may have arisen in these forms by duplication from the diploid, and have become modified in later generations by crossing-over, non-disjunction, and hybridization, until a single individual only possesses one to three quadrivalent sets of homologous chromosomes. Tetraploid roses may have arisen more than once on this continent by duplication in more or less sterile diploid hybrids. This is known to have occurred in the case of *Primula kewensis* (DIGBY 17), in *Nicotiana* (CLAUSEN and GOODSPEED 10), in *Raphanus* \times *Brassica* hybrids (KARPECHENKO 37), and in *Rosa wilsoni* (HARRISON and BLACKBURN 5). WINGE (66) believes that lack of harmony between chromosomes from two parents, even when their numbers are the same, may lead to reduplication, giving tetraploids from hybrid diploids. From his work on *Viola*, CLAUSEN (9) concludes that such doubling is important in biological evolution, and will produce "biologically stable types." Duplication, on the other hand, which produces true tetraploidy with quadrivalent sets of homologous chromosomes, he believes to be of little consequence in phylogeny, such types being unstable and likely to revert to the original diploid state.

The possibility that a tetraploid form with all the chromosomes pairing might be produced from a cross between a diploid and a hexaploid among the Cinnamomeae should not be entirely overlooked, even though this would involve pairing between the chromosomes from one parent, a condition that has not been observed in *Rosa*. LONGLEY (43) found that in a cross between *Vaccinium corymbosum* (haploid number of 24) and *V. virgatum* (with $x=36$), the F_1 hybrid occasionally showed as many as thirty pairs at diakinesis, although the hybrid was highly sterile.

MORPHOLOGICAL CHARACTERISTICS AND DISTRIBUTION
OF TETRAPLOIDS

It soon became noticeable among our living collection that nearly all the North American tetraploid species are characterized by the ability to produce flowers terminally in corymbs on the season's turions, a behavior peculiarly adapted to a prairie habitat where the aerial stems are likely to be frozen down in winter and burned off by fire in summer. They are also characterized by their long flowering period, some of them continuing to bloom after ripe fruit is developed, a performance never observed in any of the other native groups, although exhibited by *R. rugosa* Thunb. *R. acicularis*, however, will occasionally have a second blooming late in a mild autumn.

I had hoped that the ability to flower on the season's turions would prove to be a criterion for tetraploidy in American roses, and for the most part this is true. In *R. virginiana*, the largest of the eastern tetraploid species, this ability is only weakly developed and some plants will not produce flowering turions every year. It is very common in *R. californica*. *R. ratonensis* Erlanson in some ways resembles *R. woodsii*, but can immediately be distinguished from that species by its strong turions bearing terminal corymbs. It is a tetraploid.

The most unexpected exception to this generalization was the discovery of a diploid specimen of *R. subserrulata*, a possible hybrid origin of which has been previously considered.

Another exception to this character being confined to tetraploids is *R. foliolosa* Nutt., a diploid species of the Carolinae from Texas and Oklahoma. This is a low semi-herbaceous species that bears very little wood over 2 years old. It flowers in small corymbs, terminally on the annual shoots. If this species originated as a haploid from a tetraploid form and still retained the tetraploid "ever-blooming" characteristic, it would from its southern distribution be an argument in support of HURST's theory of the loss of extra septets of chromosomes from polyploids in southern habitats. It is a very distinct species, its narrow numerous leaflets and small (sometimes curved) prickles resembling, if anything, diploid *R. palustris*, whose western limit is well to the east of the range of *R. foliolosa* (text fig. 1), rather than any form of tetraploid *R. carolina*.

Our collection does not contain any mature *R. nitida* Willd. This species was reported as diploid by TÄCKHOLM, and it may also possess the ability to flower on the season's turions, although this is not mentioned in descriptions and can seldom be discovered from herbarium specimens. It will be of interest to discover whether the other dwarf species, *R. gamella* Willd. and *R. nanella* Rydb., also Carolinæ with limited ranges in the northeastern Atlantic region, are diploid or tetraploid. *R. relicta* Erlanson, a dwarf form from Illinois, and the low growing *R. bushii* Rydb. are both tetraploid. The third exception is the case of a dwarf plant from Calgary, Alberta (no. 3571). After three years in the garden it is little more than a foot high, a small branched woody bush resembling in habit the specimen of *R. subglaucæ* from Alberta (no. 3492). The former plant agrees with the description of *R. alcea* Greene. It grows very slowly and has produced no turions. The dwarf *R. subglaucæ* has produced one or two flowering turions. Both these specimens are tetraploid.

With the exception of *R. virginiana*, in the northeast, and of *R. californica*, the North American tetraploid roses are of low stature, usually under a meter. The tetraploid species belonging to the section Cinnamomeæ are more specialized in habit and inflorescence type than the related diploid forms, and are perhaps of more recent origin. The setaceous stems and numerous leaflets are considered by BOULENGER (7) as relatively primitive characteristics in *Rosa*, but they almost always appear on young turions, and would be expected on plants with a semiherbaceous habit. Some of these forms, more particularly *R. suffulta* and its allies in the Mississippi region, have a markedly semiherbaceous habit, which appearing in a frutescent group is generally considered a more highly evolved condition (SINNOTT and BAILEY 56). BEWS (3) points out that the stream bank and swamp habitat is one of the primitive unchanging habitats of angiosperms, and that the grassland habitat is a relatively recent one; hence he maintains that plants adapted to the latter are derived and not primitive forms. As already stated, the North American diploid roses are characteristic of waterways (as are the hexaploids), while the tetraploids belong to the prairies and dry uplands.

From every point of view the tetraploid forms seem to be more

highly evolved and of more recent origin than any other form in the group of Cinnamomeae-Carolinae in America.

Among the tetraploid Carolinae, *R. virginiana* stands out as the most robust and fruticose type. This species seems to be confined to the northeastern Atlantic region, although a whole series of forms can be found between it and the typical low weedy *R. carolina*, as well as between this latter species and the tetraploid Cinnamomeae of the *R. suffulta* complex. All these types flower almost synchronously when grown together. The significance of this will be considered in the discussion of phenology in *Rosa*. The tetraploid Carolinae have a very extensive north and south range, from the St. Lawrence to northern Mexico. It is noticeable that the glandular types of the *R. carolina* complex preponderate in the southern part of its range; this is also true for the western diploid *R. woodsi* complex, which has an even greater north and south range than the Carolinae, stretching from British Columbia to Chihuahua.

HEXAPLOID TYPES

All North American forms of *R. acicularis* examined, except one individual from Alaska, showed twenty-one pairs of chromosomes at metaphase at the first meiotic division in the microsporocytes (fig. 63). This species is typically boreal and alpine, and SCHNEIDER (54) was without doubt in error in attributing a Texan specimen to it. Specimens collected in the Sangre de Cristo Mountains of southern Colorado by C. O. ERLANSON grew at altitudes between 8000 and 13,000 feet; they are hexaploid and agree with RYDBERG's descriptions for *R. butleri* and *R. underwoodii* (fig. 64). After having examined several thousand herbarium specimens of North American roses, I have seen no specimens belonging to the *R. acicularis* complex from farther south than the mountains of southern Colorado.

As has been found by others, *R. nutkana* Presl. is also hexaploid, as are the allied species *R. spaldingii* Crép. (fig. 65) and *R. macdougali* Holz. *R. engelmanni* S. Wats., which is somewhat intermediate between *R. acicularis* var. *sayiana* and *R. nutkana* in the northern Rockies, is also hexaploid. The hexaploid forms exhibit very few irregularities in meiosis. Both divisions are usually almost schematic in regularity. Extrusion occurs (fig. 64); delayed pairing at di-

akinesis and lagging chromosomes have only been observed in one or two cases; polyspory is rare (fig. 66); and pollen examination reveals a very low percentage of poor pollen, in all cases less than 10 and often under 5 per cent.⁶

The *R. acicularis* forms are the first of the American species to flower in the spring, and the pollen mother cells are frequently damaged by frost at the end of May. They are followed in about a week by *R. nutkana* and its allies. In all these species the buds are relatively large (8-10 mm. long) when pollen formation takes place. Although these forms show few cytological disturbances, it is evident from the variations in cultures that they hybridize freely among themselves. Neither is artificial hybridization between hexaploid forms difficult. The hexaploids are not larger plants than the diploid Cinnamomeae.

Just as in the region of the Great Lakes, botanists have always found difficulty in distinguishing some forms of *R. blanda* from *R. acicularis*; so in the Rocky Mountain region there are many forms intermediate between *R. nutkana* and *R. acicularis*. In the case of the two latter species the flowering times are more nearly approximate, and the chromosome complements of the two species are alike, thus making chances for successful crossing greater. I suspect that *R. engelmanni* is a product of such a cross, and hope to produce it artificially in the garden. Hexaploid species have been crossed successfully to diploid forms. Crossing tetraploids with hexaploids has not been attempted, for, although their ranges overlap in the eastern foothills of the Rocky Mountains and in the northern great plains region, the wide difference in time of flowering between the two groups almost precludes this cross in the field.

As HARRISON and BLACKBURN (27) have pointed out, the hexaploid condition probably arose from the union of a tetraploid with a diploid gamete followed by duplication in a triploid hybrid. The greatest concentration of hexaploid types on this continent seems to be in the northern Rocky Mountain region, and their distribution is continuous in all directions from thence. No hexaploids have been

⁶ I wish to express my thanks to Dr. REHDER and Mr. JACK for extending to me the privilege of collecting cytological and herbarium material from the interesting hexaploid roses in the Arnold Arboretum collection in 1926.

found outside the continuous range of *R. acicularis* in regions where diploid and tetraploid forms both occur; that is, in the northwestern part of the great plains, and in the United States east of the Mississippi (text figs. 1, 2); so that it would appear as though the hexaploid type has not arisen more than once, if at all on the American continent. There has not been discovered a hexaploid in the section Carolinae. The hexaploid types of Cinnamomeae may have entered America from northeastern Asia and spread south and east over the continent.

Meiosis in ovule.—Meiosis has not been observed in the megasporocyte of any hexaploid type. Stages in fertilization as well as a normal two-celled embryo have been observed in *R. acicularis* var. *lacorum* from northern Michigan (text fig. 3). Castrated buds have continually failed to give any fruit.

R. nutkana and related species.—The diploid *R. pisocarpa* and the tetraploid *R. californica* both show some morphological resemblance to *R. nutkana*. This fact has been evident to students of the Pacific Coast roses. WATSON (63) placed *R. californica* and *R. pisocarpa* in the same subdivision. In determining TÄCKHOLM's material, ALMQUIST identified the diploid "*R. californica*" specimens from Lund and Kew as varieties of *R. nutkana*, and gave the same treatment to the *R. pisocarpa* specimens. It is fairly certain that former students of the Pacific Coast roses have usually had only small series of specimens at their disposal, and have misunderstood the species as did CRÉPIN (RYDBERG 48). Nevertheless there is a superficial morphological resemblance between these species in some respects, just as there is between *R. acicularis*, *R. blanda*, and *R. suffulta* in the northern great plains region, although one would never confuse them in a living collection. There is thus some evidence supporting the suggestion of HARRISON and BLACKBURN (27) that the North American Cinnamomeae species have a dual origin, one group of species arising from *R. nutkana*. Their "neat proof," however, from the presence of a specific rose gall insect is unfortunately not justified by the work of KINSEY (40) on which they base it.⁷ It has

⁷ Dr. KINSEY informed me that it was not his intention to affirm that *Diplolepis bassetti* is "confined to *R. nutkana*." The following are extracts from his letters in 1926: "The host record which I gave for *Diplolepis bassetti* should have been applied only to the type material of variety *bassetti*. . . . I have no determinations of any sort



FIG. 1.—Map showing distribution of Carolinae: area of diploid species delimited by dots; tetraploids by crosses

seemed to me for some time that the two series of species groups (1) *R. nutkana* (hexaploid), *R. pisocarpa* (diploid), *R. californica* (tetraploid), in the far west and (2) *R. acicularis* (*R. sayi*) (hexaploid), *R. blanda* (diploid), *R. suffulta* (tetraploid), or *R. acicularis*, *R.*



FIG. 2.—Map showing distribution of American Cinnamomeae: area of hexaploid species delimited by crosses; diploids by dots; tetraploids by wavy line.

woodsi, *R. arkansana* in the Mississippi region, are analogous in the origin and relationship of the contained forms. If the chromosome numbers in our species have become reduced in phylogeny, as HURST claims, this has apparently not been in a simple descending series, and there seems to be no reason to suppose that the process which

on the roses from which I secured additional material of the same variety. . . . The only material which I have shows nothing but a remnant of a stem without leaves. . . . I have no notion what rose this species (*Diplolepis bassetti*) occurs on and I am very much inclined to believe that it occurs on more than one rose in this wide range (i.e. Oregon, Idaho and Utah)."

produces forms related to the polyploid species but with smaller chromosome numbers is anything other than hybridization, followed by the loss of unpaired chromosomes, as previously suggested under diploid species.

OCTOPOLOID TYPE

A large bush of *R. acicularis* from Europe at the Arnold Arboretum, which was grown from seed sent from the Royal Gardens at Kew, has only twenty-one pairs at diakinesis, as found also by PEN-

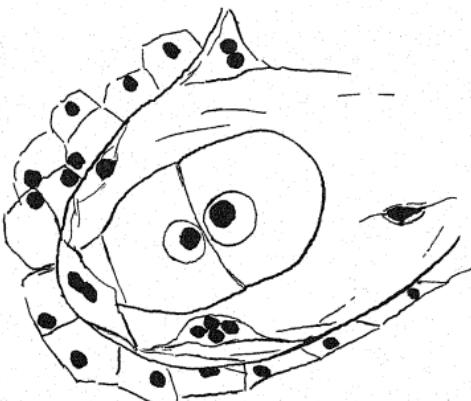


FIG. 3.—Two-celled embryo of *R. acicularis* var. *lacorum* no. 6007 (hexaploid)

LAND (46), and not twenty-eight as in European material from Sweden and Russia examined by TÄCKHOLM.

An individual from a collection sent by Professor C. H. MORGAN from Fairbanks, Alaska (accession no. 6447) was found to be an octoploid with twenty-eight pairs at diakinesis (figs. 67, 68). Meiosis in this form is regular throughout and no polyploidy has been observed. The plant possesses very glandular foliage and narrow pendant hips, and fits well into *R. acicularis* var. *lacorum* as described from the Great Lakes region (ERLANSON 18), differing from that form only in its earlier flowering period. Herbarium specimens of the European forms of *R. acicularis* were examined in the Crépin Herbarium at Brussels in 1925, and no distinguishing morphological character could be found, that would hold out in a series of speci-

mens, to distinguish them from North American types of the species. All that can be said is that glands on the foliage are far commoner in American than in European types, in which glands are exceptional. There does seem to be some difference in habit, however, the European bushes that I have seen in botanical gardens branching more freely and reaching a greater height than our specimens. The Alaskan plants in our collection thus far show no marked habit differences from the other American forms. This, therefore, is the second case in which two individuals have been discovered apparently belonging to the same species yet possessing different chromosome numbers (the other case is mentioned under the tetraploid *R. sub-serrulata*).

The octoploid *R. acicularis* has exceptionally few deformed pollen grains. In 400 grains examined not one shrunken and transparent grain was observed, the only irregularity exhibited in the pollen being 3.75 per cent of distorted and angular (though plump) grains amongst the normal ones.

Polyspory and apomixis

POLLEN EXAMINATION

The first published report of pollen examination in American rose species is that of CRÉPIN (15), who reports "pollen pur" in all the forms examined, except in the case of *R. californica*, the pollen of which he found "tantôt complètement pur, tantôt mêlé d'environ $\frac{1}{2}$ de grains atrophiés." He thought originally that the presence of dwarf grains gave "une précieuse indication dans le cas où une forme est soupçonnée d'hybridité," but was constrained to modify his views on account of the pollen sterility present in the Caninae. He found that the amount of bad pollen varied in different flowers from the same bush, and probably also from year to year. COLE (12) reported shriveled grains among the pollen of several American rose species but did not give actual counts. HARRISON and BLACKBURN (27) showed that the amount of bad pollen among roses of the Caninae varies from 10 to 100 per cent.

In order to examine pollen, anthers were taken from buds just before the petals opened, and stored over calcium chloride in a desiccator. The pollen was examined dry under the microscope and

the percentage of shriveled grains in a sample of about 500 was calculated. As shown in table IV, the octoploid *R. acicularis* and all

TABLE IV
PERCENTAGE OF STERILE GRAINS IN POLLEN OF AMERICAN ROSES

| SPECIES | ACCESSION NO. | ORIGIN | PERCENTAGE STERILE POLLEN |
|--|---------------|-------------|---------------------------|
| <i>Diploid</i> | | | |
| <i>R. gymnocarpa</i> | 4006 | Idaho | 9 |
| <i>R. ultramontana</i> | 5292 | Washington | 4.7 |
| <i>R. salicetorum</i> | 3530 | Idaho | 38.0 |
| <i>R. woodsi</i> | 5560 | Montana | 9.0 |
| <i>R. fendleri</i> | 2938 | Colorado | 25.6 |
| <i>R. blanda</i> | 2895 | Iowa | 10.0 |
| <i>R. blanda</i> | 3502 | Illinois | 52.0 |
| <i>R. blanda</i> var. <i>hispida</i> | 2681 | N. Dakota | 4.3 |
| <i>R. blanda</i> var. <i>hermanni</i> | 2686 | Michigan | 6.0 |
| <i>R. blanda</i> var. <i>glandulosa</i> | 3753/9 | Michigan | 17.6 |
| <i>R. blanda</i> var. <i>glandulosa</i> | 5/110 | Michigan | 25.0 |
| <i>R. schuetteana</i> | 5891 | Michigan | 8.3 |
| <i>R. michiganensis</i> | 5890 | Michigan | 3.6 |
| <i>R. palustris</i> | 4412 | Michigan | 10.0 |
| <i>R. palustris</i> | 5714 | Michigan | 4.8 |
| <i>R. palustris</i> | 8626 | Ohio | 9.5 |
| <i>Tetraploid</i> | | | |
| <i>R. californica</i> | 5849 | California | 26.0 |
| <i>R. aldersoni</i> | 5367 | California | 22.0 |
| <i>R. suffulta</i> | 2682 | N. Dakota | 15.5 |
| <i>R. suffulta</i> | 3001 | Kansas | 29.0 |
| <i>R. rudiiscula</i> | 3501 | Illinois | 25.8 |
| <i>R. bushii</i> | 3080 | Missouri | 26.0 |
| <i>R. relecta</i> | 8320 | Illinois | 56, 77 |
| <i>R. carolina</i> | 5716 | Michigan | 25.4 |
| <i>R. carolina</i> | 2946 | New York | 9.5 |
| <i>R. virginiana</i> | 6071 | Maine | 24.4 |
| <i>R. virginiana</i> | 4651 | Connecticut | 10.0 |
| <i>Hexaploid</i> | | | |
| <i>R. spaldingii</i> | 5298 | Washington | 8.0 |
| <i>R. spaldingii</i> | 5549 | Washington | 8.6 |
| <i>R. butleri</i> | 8271 | Colorado | 0.0 (8.3% angular grains) |
| <i>R. underwoodii</i> | 8270 | Colorado | 8.3 |
| <i>R. engelmanni</i> | 3721 | Wyoming | 10.8 |
| <i>R. acicularis</i> var. <i>lacorum</i> | 6008 | Michigan | 8.4 |
| <i>Octoploid</i> | | | |
| <i>R. acicularis</i> | 6447 | Alaska | 0.0 (3.6% angular grains) |
| <i>Triplloid</i> | | | |
| <i>R. sp. indet.</i> | 2872 | N. Carolina | 100.0 |
| <i>R. sp. indet.</i> | 5590 | Connecticut | 97.0 |
| <i>R. sp. indet.</i> | 2949 | New York | 92.7 |

hexaploid forms examined had about 10 per cent or less of shriveled pollen. Several diploid forms have less than 10 per cent of shriveled grains. *R. salicetorum* and *R. fendleri* showed 38 and 25.6 per cent

respectively. There is great variation in the amount of sterile pollen in different individuals of *R. blanda* and its varieties, varying from 4.3 to 52 per cent. Most of the tetraploids show from 15 to 25 per cent of bad pollen. Considerable difference is shown between two individuals of the same species in *R. carolina* and in *R. virginiana*. *R. relicta* Erlanson has the highest amount of sterile pollen of any form examined (except the triploids), one bud having 56 and another 77 per cent of bad pollen.

These counts demonstrate that many of our native rose species are partially sterile, a characteristic often associated with hybridity. It is very interesting to find that, among forms with all pairing chromosomes, the higher polyploids show the least amount of sterile pollen, while the tetraploids show the most; the diploids occupy an intermediate position in this respect, showing much variation.

CASTRATION EXPERIMENTS

TÄCKHOLM (59) has given an account of the castration experiments carried out in Europe on *Rosa*. HARRISON and BLACKBURN (27) report that several of the English Caninae are facultatively apomictical. HURST (30) reported the production of normal achenes after castration in *R. gallica* and other garden forms. Castration has been carried out on several North American species during the past three years, and in only two cases were fruits obtained subsequently. These were both in 1927 on plants of *R. blanda* from northeastern Illinois; plant 8321 on which two buds were castrated gave one small hip containing two achenes; six buds were castrated on plant no. 3502 and every one set a plump hip filled with achenes. In castrating, the androecium and the stigmas were removed with a razor blade but the buds were not bagged. The case of 8321 may have been due to a failure to remove all the stigmas; the case of 3502 is significant when its high percentage (52 per cent) of bad pollen is considered. ERNST (21) maintains that polyspory and apomixis are consequent upon hybridization.

Phenology in relation to phylogeny

Taxonomists formerly seem to have agreed that *R. acicularis* is the most primitive member of the Cinnamomeae (BOULENGER 7), and my observations tend to confirm this. The tetraploid forms

seem to be the most highly evolved and specialized of the American Cinnamomeae, while the related diploids are intermediate between the hexaploids and tetraploids. There appear to be two distinct series of hexaploid, diploid, and tetraploid forms, with ranges on the western and eastern sides of the Rocky Mountains respectively. In each series the hexaploids are boreal and alpine in distribution, the tetraploids temperate and southern (text fig. 2).⁸ In both of these series the diploids occupy a middle position in the southern part of the range of the series as a whole, and are intermediate in flowering time between the early-flowering hexaploid species and the later-flowering tetraploids.

There is some evidence that the phenological procession in the flora of the northern hemisphere, taken by and large, gives some clue to phylogenetic relationships, the vernal forms being the most primitive, as suggested by HARSHBERGER (28) and maintained by ILLITSCHEVSKY (34) at the International Congress of Botany in 1926 at Ithaca. This principle may also help in an understanding of the phylogeny of the species in some large boreal genera.

Phenological notes have been taken on the University of Michigan rose collection for three years, and each species complex is found to have a specific and characteristic flowering period. When taken in relation to each other each group of forms comes into flower in a definite order. In fact the "flowering time" of any individual is often a very good specific character, a fact to which CRÉPIN (14) first called attention. A collection of plants belonging to *R. blanda* var. *hispida* which was received from Manitoba (no. 5771) contained one plant which did not differ markedly from the others in habit, yet it began to bloom about 10 days later. Cytological examination revealed that the earlier blooming form was diploid while the latter one was tetraploid. Upon closer examination the latter specimen was found to have 7-9 glabrous leaflets; the petals were pale salmon

⁸ In text figs. 1, 2, and 4 the distribution areas have been plotted whenever possible from specimens seen by the writer. An "!" means that a specimen has been verified from that particular region. Doubt about the occurrence of a certain group is indicated by an "?" immediately following the number, which denotes the chromosome constitution of the group. The limits of the areas of distribution are approximately correct, although they may have to be extended in some directions when more specimens have been studied.

color, not rose pink as in the other plants; and the hips were larger with a slight neck. I have placed it under *R. arkansana* Porter. Although we have several collections of each species complex from different parts of its range, a hexaploid type from the mountains of Montana flowers synchronously with hexaploid forms from the mountains of southern Colorado and from central Michigan; even as tetraploid forms from North Dakota and Alberta bloom no earlier than those from Missouri. The many varieties of *R. blanda* obtained from stations all over its range all come into flower during the same week. The octoploid *R. acicularis* from Alaska is the first of the American plants to come into flower; in 1926 it bloomed a week before any of the hexaploid forms of this species.

Possible origin of polyploid series

CINNAMOMEAE

The conclusion that hybridization between many of our wild forms is general and of common occurrence in nature is supported by: (1) the successful production of good achenes by artificial crossing of different species with the same chromosome number, of diploids with hexaploids and of diploid with tetraploid forms; (2) the marked variation in the progeny obtained from seeds taken from individual wild plants in the field; (3) the large and variable percentage of sterile pollen present in many wild individuals; (4) the meiotic irregularities similar to those found by TÄCKHOLM in plants with a balanced number of chromosomes but which were known to be hybrids. Furthermore, it appears as though all crosses between parents with unlike chromosome number are not sterile. The evidence is strong for the theory of HARRISON and BLACKBURN of "the hybrid origin of orthoploid series in *Rosa* paralleling that in other plant genera." If this premise be accepted, it is possible to postulate an origin for the polyploid series which we find in the North American Cinnamomeae (table V). As COCKERELL (11) pointed out, the octoploid and hexaploid types were probably built up by duplication from primitive diploid roses, and although HURST does not mention this in his preliminary paper (31), this is also his idea of their origin.

The northern limit of hexaploid types on this continent is at present unknown, as is the southern limit of the octoploid *R. acicularis*. It is reasonable to suppose that both types reached America

TABLE V

Phylogenetic diagram

AMERICAN CINNAMOMEAE

Ancestral diploid forms by duplication and
hybridization → higher polyploids

R. acicularis (octoploid) \times *R. nutkana* (hexaploid)

↓ (or related form)

Heptaploid hybrid

↓

Later generations by elimination of univalent chromosomes

↓

R. engelmanni, *R. acicularis sayiana*, *R. acicularis bourgeauiana*
(hexaploid types)

R. nutkana (hexaploid) \times (diploid) Generalized ancestral type of

↓ *R. woodssii* group

Tetraploid hybrid

7 bivalents and 14 univalents at diakinesis

↓

Later generations by elimination of univalents

↓

R. pisocarpa (diploid) \times diploid form

↓

diploid hybrid

↓

by duplication

↓

R. californica (tetraploid)

R. acicularis sayiana (hexa.) \times *R. woodssii* ancestor (diploid)

↓

Tetraploid hybrid

↓

Later generations by elimination of univalents

↓

R. blanda (dipl.) \times *R. macounii*, *R. fendleri* (diploids)

↓

diploid hybrid

↓

by duplication

↓

R. suffulta, *R. arkansana* (tetraploid) \times *R. blanda*

↓

triploid hybrid

↓

by two divisions of univalents

↓

R. relicta (tetraploid)

TABLE V—Continued

DIAGRAM OF POSSIBLE ORIGIN OF SOME CAROLINAE

R. virginiana (tetraploid) \times *R. palustris* (diploid)

triploid hybrid

↓
by elimination of univalents

R. nitida (diploid)

↓
by two divisions of univalents

tetraploid form \times *R. virginiana*

(tetraploids) *R. carolina*, *R. lyoni*, *R. serrulata*

from the northwest, coming from northeastern Asia, as suggested for the hexaploids by HARRISON and BLACKBURN (27).

In 1927, pollen from the octoploid *R. acicularis* was collected May 24 and kept in a desiccator until June 14; it was then used to pollinate four buds of *R. nutkana* from Idaho. Large hips were set, containing 16, 17, 22, and 22 achenes respectively. Seedlings from this cross would be expected to be heptaploid with forty-nine somatic chromosomes, and to show twenty-one pairs and seven univalent chromosomes at diakinesis. In F_2 and later generations a fertile hexaploid race might be reestablished by the loss of the unpaired chromosomes by lagging at meiosis, as has been found to occur in wheat hybrids (SAX 51, 52, 53; KIHARA 38, 39). These hexaploid descendants should possess some of the characteristics of each parent. Such a cross may well have been the origin of the American hexaploid forms such as *R. engelmanni*, *R. acicularis* var. *sayiana*, and *R. acicularis* var. *bourgeauiana*.

A good yield of achenes was obtained by crossing *R. woodssii* (diploid) with *R. spaldingii* (hexaploid), and also from crossing *R. salicetorum* (diploid) with *R. nutkana* (hexaploid). Some such cross, followed by an elimination of unpaired chromosomes in subsequent generations, may have given rise to the diploid *R. pisocarpa*. Some related sterile diploid form, by duplication, may have originated the tetraploid *R. californica*. Such a phylogeny does not seem too far-fetched, and would account for the resemblances already noted between *R. nutkana*, *R. pisocarpa*, and *R. californica*.

A hexaploid *R. acicularis* crossing with some member of the

diploid *R. woodsii* group, by elimination of unpaired chromosomes in later generations, could be imagined to have produced the *R. macounii-fendleri* group of diploid forms. Some one of these, crossing with *R. blanda* (or other diploid), may have resulted in a sterile diploid form, which by duplication gave rise to a fertile tetraploid race, the *R. suffulta-arkansana* group, which latter group of forms always seemed to CRÉPIN to be a variety of *R. blanda*.

The distribution map of the American Cinnamomeae (text fig. 2), although only approximate, brings out the fact that the diploid and hexaploid forms occur in that area of the continent which was occupied by the Pleistocene ice sheet (COLEMAN 13), the diploid forms having spread appreciably south of this area only in the western mountainous regions and in the foothills. The distribution area of *R. californica* lies well to the south of the glaciated area, and that of the tetraploid *R. arkansana* group is, for the most part, also to the south of it. The latter group is spreading rapidly both northward and eastward, however, having been found in the last few years both in southern Michigan and in northern New York.

The general area of distribution of *R. blanda* (text fig. 4) contrasts with that of the other Cinnamomeae in being northeastern and in not reaching the Rocky Mountains, although many closely related forms are found there. Whether *R. blanda* was already on this continent before the Pleistocene ice sheets advanced, and whether it therefore, upon their final withdrawal, advanced northward and westward, finally meeting the hexaploid and diploid forms coming in from the extreme northwest, is an interesting speculation.

CAROLINAE

The generalized distribution map of the Carolinae (text fig. 1) shows that these forms occupy the southeastern part of the continent, chiefly that region not occupied by the Cinnamomeae. In the case of the Carolinae, the diploid and tetraploid types occupy almost the same area, the tetraploids going farther into the southwest. The Cinnamomeae and Carolinae overlap chiefly in the Great Lakes region and in the central part of the Mississippi Valley, where tetraploid forms belonging to both sections are found. It is somewhat certain that these tetraploids hybridize and give rise to intermediate

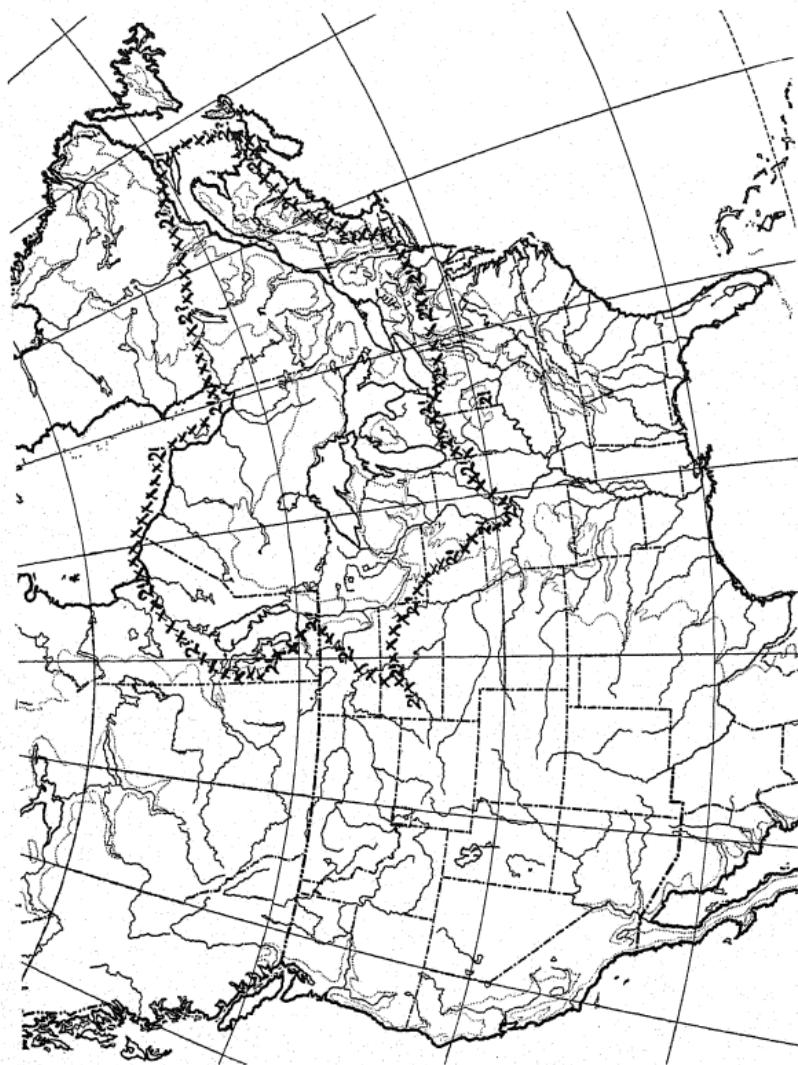


FIG. 4.—Map showing distribution area of *Rosa blanda* Ait.

forms such as *R. rudiuscula* Greene, *R. bushii* Rydb., and *R. juncta* Rydb.

As stated elsewhere (ERLANSON 19), *R. palustris* and *R. blanda* appear to cross in the northern Lake Michigan region and to give stable forms. *R. virginiana* may be an ancient tetraploid type, and is typically limited to the northeastern Atlantic region. *R. palustris* crossed with *R. virginiana* would give a triploid hybrid, which would probably be sterile. Since no fertile hexaploid forms are known in this group, a fertile tetraploid may have been reestablished from such a sterile triploid by all the unpaired chromosomes dividing at both meiotic divisions, as in our accession no. 2949 from Ithaca, N.Y., and this tetraploid may have been the ancestral form of the complex of *R. carolina*, *R. lyoni*, *R. serrulata*. Specimens of *R. palustris* with gland-compound teeth have been observed in herbaria.

No sterile wild diploid type has yet been discovered, although there is the possibility that they exist and produce fruit apomictically.

The fact that vigorous forms, originally of hybrid origin, may spread beyond the distributional limit of at least one parent is seen in the case of *R. blanda* var. *glandulosa*, which is found farther south along the shores of Lake Michigan than *R. acicularis*. An alternate origin of the hexaploid forms on this continent from a cross between a tetraploid and diploid followed by duplication does not seem feasible from a consideration of the distribution data.

Hurst's theory of differential septets

Owing to the interest which HURST's theory of the origin of forms with lower numbers from higher polyploids in *Rosa* has aroused, the evidence for and against his interpretation, taken from findings in American material, follows.

Evidence favoring hypothesis

1. Octoploid *R. acicularis* is apparently arctic, with the hexaploid *R. acicularis* types boreal and alpine in distribution. The resemblance between the two types could be interpreted to mean that one "double septet" (fourteen homologous chromosomes) does not affect the octoploid plant and could therefore be dispensed with if eliminated by extrusion.

2. Two forms occur in southern stations, with flowering times and habit characteristic of tetraploids, yet having only fourteen chromosomes. These are *R. foliolosa* and the diploid *R. subserrulata*. The latter appears to be almost identical morphologically with the tetraploid *R. subserrulata*, and both it and *R. foliolosa* seem to be "haploid tetraploids" rather than simply primitive diploids. If this were so these forms could be interpreted as having arisen from tetraploid ancestors by the loss of half of the chromosomes ("one double septet") in southern latitudes.

Evidence against hypothesis

1. Neither HARRISON and BLACKBURN nor I have been able to discover any convincing cytological evidence for distribution of the chromosomes in sets of seven during reduction division in *Rosa*, as would be required by HURST's theory if the "differential septets" are to remain together as compound linkage groups. On the contrary, the cytology of many American forms demonstrates the independent and often irregular distribution of chromosomes at meiosis. In the same plant varying numbers of bivalent groups are present in different cells at first metaphase, and varying numbers of the univalents may be split at first anaphase. The fact that all gametes resulting from irregularities in meiosis, or containing extra chromosomes, are not sterile is shown by the existence of fertile, vigorous aneuploid plants.

2. Chromosome extrusion is frequently observed in the microsporocytes of *Rosa*, but the number of chromosomes thus lost from the cell seems to be arbitrary and variable, and is probably due to disturbances consequent upon hybridization.

3. It is very doubtful that all crosses between different "simple septet" species (diploids) give sterile diploid hybrids. Intermediate types between *R. palustris* and *R. blanda* (which are surely different "simple diploid species") in northern Michigan, such as *R. schuetiana* and *R. michiganensis* (ERLANSO¹⁹), have been found to be diploid and have very little sterile pollen.

4. If there are races within some species with different chromosome complements which are indistinguishable morphologically, as seems to be the case in *R. acicularis* and *R. subserrulata*, then we

cannot base a classification of the genus on the "numbers of somatic septets of chromosomes present in the species," as HURST proposes to do.

Summary

1. In this study 107 wild roses from known stations in North America were examined cytologically. The majority of the specimens fall into the three classes, diploid, tetraploid, and hexaploid, seven being the basic number. One specimen was octoploid, two were triploid, and three aneuploid with the somatic number not a multiple of seven.

2. Measurements show that the cells are larger in the polyploid forms.

3. The diploid American roses are robust plants, larger as a rule than the related polyploid species. Many of them exhibit irregularities during meiosis. In some individuals incomplete pairing at diakinesis, lagging chromosomes, and polyspory are frequent; these are believed to be spontaneous hybrids produced by the crossing of different species, or the descendants of such hybrids.

4. It is concluded that crossing occurs in the field between different diploid species in *Rosa*, giving rise to at least partially fertile diploid offspring.

5. Three fertile aneuploid plants, two with sixteen somatic chromosomes and one with fifteen, have been found among cultures of diploid plants. One such culture exhibits a great deal of variation and appears to be a later generation of a cross between the diploid *R. blanda* and the hexaploid *R. acicularis* of Michigan. Such a cross would give, in the first generation, tetraploid hybrids with seven pairs and fourteen unpaired chromosomes. During meiosis in these hybrids the unpaired chromosomes probably lag on the spindle, giving some fertile gametes with only seven or eight chromosomes, which by self-fertilization give rise to fertile diploid or aneuploid plants.

6. Two triploid plants have been examined cytologically. Both have seven paired and seven unpaired chromosomes at first reduction division. One exhibits the "*Drosera*-type" of meiosis and is sterile. The other has the "*Rosa*-type" of meiosis, in which the seven univalent chromosomes divide twice, making it possible for

some spores to receive fourteen chromosomes, although this is prevented in many cells by lagging on the spindle. This latter plant is not apomictical yet it sets good fruit. Reduction in the megasporocyte of the fertile triploid has not been observed. The plant may be a starting point for a race of triploid roses analogous cytologically to the pentaploid *Caninae*, or it is able to give either tetraploid or diploid offspring by self-fertilization. A third plant with over 90 per cent of the pollen sterile is suspected of being a triploid hybrid from *R. palustris* \times *R. virginiana*.

7. *R. californica* and related species were found all to be tetraploid with fourteen pairs at diakinesis.

8. The tetraploid species examined cytologically nearly all exhibit irregularities during meiosis and also polyspory. They thus resemble hybrid forms in their cytological behavior.

9. Both tetraploid and diploid races morphologically similar were found in *R. subserrulata* Rydb.

10. The tetraploid forms often exhibit some rings of four chromosomes at diakinesis, but none have been found that are completely quadrivalent. It is concluded that they originated by duplication of diploid hybrids and have hybridized with each other.

11. The tetraploids are characterized by being able to flower on the season's turions and by a long flowering period. Many of them are semi-herbaceous or dwarf, and are believed to be recent or derived forms adapted to prairie and dry upland habitats.

12. *R. nutkana* and related species, and all but one of the specimens of *R. acicularis* and its varieties, were found to be hexaploid, with twenty-one pairs at diakinesis.

13. Hexaploids exhibit few meiotic irregularities.

14. All hexaploids in America apparently occupy one continuous range, and it is thought that they have not originated on this continent from a sterile triploid hybrid by duplication, but that they migrated from Asia as hexaploids spreading southward and eastward.

15. There are two parallel series of American Cinnamomeae, each consisting of hexaploid, diploid, and tetraploid types: (1) on the Pacific Coast *R. nutkana*, *R. pisocarpa*, and *R. californica*; (2) in the Mississippi Valley *R. acicularis*, *R. blanda*, and *R. suffulta*.

16. A specimen of *R. acicularis* from Alaska was found to be octoploid with twenty-eight pairs at diakinesis. The plant does not differ markedly from the hexaploid variety *lacorum*, but is distinguished by coming into flower earlier. It has pure pollen.

17. Examination showed that the hexaploids have a small proportion (under 10 per cent) of sterile pollen. Some diploid specimens have almost entirely good pollen while others have 10-15 per cent sterile grains. Most of the tetraploids showed 15-30 per cent sterile pollen, although individuals of *R. carolina* and of *R. virginiana* were found with 90 per cent of their pollen good. One tetraploid had 56-77 per cent of the pollen bad.

18. Good fruit was obtained after castration in one case only, that of a specimen of *R. blanda* (diploid) from Illinois which had 52 per cent bad pollen.

19. The flowering times of each of the large species groups are specific and characteristic when taken in relation to each other. The phenology of the species of American Cinnamomeae seems to coincide with phylogenetic relationship, those coming into flower first being the most primitive. The flowering order is: octoploids, hexaploids, diploids, tetraploids.

20. Cytological and morphological evidence support the theory of HARRISON and BLACKBURN of the hybrid origin of orthoploid series in *Rosa*. A possible origin of the North American polyploid series is worked out from this premise.

21. The presence of octoploid and hexaploid races of *R. acicularis* in America, and also of diploid and tetraploid races of *R. subserrulata*, is suggested as evidence in support of HURST's theory.

22. Cytological observations on American roses do not support HURST's theory of differential septets in *Rosa* which would require that sets of seven chromosomes separate together during meiosis. Extrusion is thought to be due partly to imperfect fixation and partly to disturbances consequent upon hybridization.

UNIVERSITY OF MICHIGAN
ANN ARBOR, MICH.

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EXPLANATION OF PLATES XVI-XIX

Except as otherwise stated, all figures were drawn with a Zeiss compensating ocular no. 20 and a 1.5 mm. apochromatic objective, with the aid of a camera lucida. They were reduced two-thirds in reproduction.

PLATE XVI

FIG. 1.—*R. bracteata* 3676, diploid; first metaphase plate, seven pairs.

FIG. 2.—*R. gymnocarpa* 4006, diploid; first meta-anaphase, seven pairs.

FIG. 3.—*R. pisocarpa* 4634, diploid; diakinesis showing three types of pairing.

FIG. 4.—*R. woodii* 8152, diploid; early first telophase.

FIG. 5.—*R. salicetorum* 3530, diploid; early diakinesis with chain of six chromosomes.

FIG. 6.—*R. granulifera* 5925, diploid; two cells at diakinesis each with six pairs and two unpaired chromosomes, exhibiting distortion of nuclei and extrusion of chromosomes.

FIG. 7.—*R. hypoleuca* 8154, diploid; diakinesis, multipolar spindle.

FIG. 8.—*R. blanda* 3942, diploid; second metaphase plates.

FIG. 9.—*R. blanda* var. *hispida* 5771 (plant B), diploid; first meta-anaphase spindle with one pair off in cytoplasm.

FIG. 10.—*R. blanda* var. *glandulosa* 3753 (plant 5/N₃), diploid; somatic prophase.

FIGS. 11, 12.—*R. blanda* var. *glandulosa* 3753 (plant 2/N₃), diploid; diakinesis nuclei with two unpaired chromosomes and rings of two chromosomes.

FIG. 13.—Somatic prophase in root tip of semihardy, sterile segregate of culture 3753; fourteen chromosomes.

FIG. 14.—Fifteen chromosomes segregate in culture 3753 (plant 9/N₁); somatic prophase in parietal cell of pollen sac.

FIG. 15.—Plant 9/N₁, culture 3753, diakinesis; six pairs and three unpaired chromosomes; majority of chromosomes from contiguous cell in cytoplasm.

FIG. 16.—Culture 3753, plant 9/N₁, first metaphase; seven pairs and one univalent chromosome.

FIG. 17.—Culture 3753, plant 9/N₁, first metaphase; four bivalents and one univalent on spindle, three bivalents in cytoplasm.

FIG. 18.—Culture 3753, plant 9/N₁, sister second metaphases with seven and eight chromosomes.

FIG. 19.—Culture 3753, plant 9/N₁, sister second metaphases each with seven chromosomes; extra chromosome (?) on surface of shrunken protoplast.

PLATE XVII

FIG. 20.—*R. acicularioides* 8261, diploid; first metaphase with five bivalents and four univalent chromosomes.

FIG. 21.—*R. acicularioides* 8261, diploid; somatic prophase in integument.

FIG. 22.—*R. pyrifera* 6610, aneuploid; diakinesis, seven bivalent and two univalent chromosomes.

FIG. 23.—*R. pyrifera* 6610, aneuploid; diakinesis, seven pairs and one smaller eighth pair.

FIG. 24.—*R. woodsii* 4477, five young microspores produced from one sporocyte (ocular no. 10, objective 1.5 mm.).

FIG. 25.—Culture 3753, plant 7/N37, four megasporae (two superimposed) each with eight chromosomes, surrounded by archeosporium (ocular no. 10, objective 1.5 mm.).

FIG. 26.—Culture 3753, plant 7/N37, sixteen chromosomes in somatic prophase in cell from chalazal region.

FIG. 27.—Culture 2872, triploid; entering first metaphase, seven bivalent and seven univalent chromosomes.

FIG. 28.—Culture 2872, triploid; first metaphase, seven bivalent and seven univalent chromosomes.

FIG. 29.—Culture 2872, triploid; sister second metaphase plates with twelve and nine chromosomes.

FIG. 30.—Culture 2872, triploid; second anaphase spindle with fourteen large chromosomes (the halves of seven original bivalents) and eight others. This nucleus received seven univalents at first telophase, one of these has split, two are dividing longitudinally at equator, one lies at one pole partly split.

FIGS. 31, 32.—Culture 2872, sterile triploid; products of two microsporocytes ten cells from one and two large cells from the other (ocular no. 10, objective 1.5 mm.).

FIG. 33.—Culture 2949, triploid; twenty-one chromosomes at diakinesis, including four or five pairs.

FIG. 34.—Culture 2949, fertile triploid; late first anaphase, seven chromosomes at each pole (originally all paired), seven univalents dividing at equator.

FIG. 35.—Culture 2949, triploid; sister second metaphase plates, with fourteen and nine chromosomes (ocular no. 20, objective 1.8 mm.).

FIG. 36.—Culture 2949, triploid; somatic prophase in nucellus.

FIG. 37.—Culture 2949, triploid; somatic prophase in stylar cell, twenty-one chromosomes.

PLATE XVIII

FIGS. 38A, 38B.—*R. californica* 5849, tetraploid; cut cell at diakinesis, twelve bivalent chromosomes, ring of three and one uncondensed chromosome.

FIG. 39.—*R. brachycarpa* 5301, tetraploid; diakinesis, twelve pairs and a ring of four.

FIG. 40.—*R. alderssonii* 5367, tetraploid; sister second metaphase plates with fourteen chromosomes each.

FIG. 41.—*R. myriantha* 3537/B, tetraploid; fourteen pairs at diakinesis.

FIG. 42.—*R. obovata* 2651, tetraploid; late diakinesis, fourteen pairs (note condensed group of four).

FIG. 43.—*R. lyoni* 2883/A, tetraploid; late interkinesis, sister nuclei each with fourteen chromosomes.

FIG. 44.—*R. carolina* 5808/B, tetraploid; late first anaphase, two pairs lagging.

FIG. 45.—*R. carolina* 5808, ring of four chromosomes from cut cell at diakinesis stage.

FIG. 46.—*R. carolina* 5808, cut cell at first metaphase, ring of four and pair of chromosomes off in cytoplasm.

FIG. 47.—*R. serrulata* (*R. carolina* var. *glandulosa*) 3938, tetraploid; somatic prophase in stylar cell with twenty-eight chromosomes.

FIG. 48.—*R. carolina* var. *litoralis* 2654; seven cells the product of one microsporocyte (ocular no. 10, objective 1.5 mm.).

FIG. 49.—*R. radiosa* 4001, tetraploid; diakinesis, twelve pairs and one group of four; seven pairs with chromosomes more closely appressed and condensed.

FIG. 50.—*R. bushii* 3080, tetraploid; second anaphase, each grand-daughter nucleus receiving fourteen chromosomes.

FIG. 51.—*R. suffulta* 2682, tetraploid; two cells at diakinesis: one (cut) showing two rings of four, two unpaired chromosomes and seven pairs; other with eleven pairs and two unpaired chromosomes being extruded into next cell and two pairs left.

FIG. 52.—*R. suffulta* 2692, tetraploid; late diakinesis showing fourteen pairs, including condensed group of four chromosomes.

FIG. 53.—*R. suffulta* 5705, tetraploid; diakinesis with fourteen chromosomes fairly closely paired, chain of six, group of four, and four chromosomes loosely grouped in pairs.

FIG. 54.—*R. suffulta* var. *valida* 4459/A, tetraploid; late first anaphase, two pairs lagging much behind the others.

PLATE XIX

FIG. 55.—*R. subglauca* 3492, tetraploid; first metaphase plate with fourteen pairs.

FIG. 56.—*R. subglauca* 3492, tetraploid; first metaphase with twelve bivalent and four univalent chromosomes.

FIG. 57.—*R. subglauca* 3492, tetraploid; end of first anaphase, showing irregular distribution of chromosomes: twelve at one pole, one near equator, fifteen (including one split) at opposite pole, one small chromosome in cytoplasm (perhaps due to a univalent having divided at first anaphase).

FIG. 58.—*R. subglauca* 3492, tetraploid; second telophase, one spindle with thirteen chromosomes at each pole and one dividing at equator, the other with thirteen at one pole and twelve at other and one lagging chromosome; also one

split chromosome and four others off in cytoplasm. Extra chromosomes resulting from some univalents dividing at both anaphases.

FIG. 59.—*R. subserrulata* 6072, tetraploid; late first anaphase, ten chromosomes at each pole, three pairs and two univalents at equator, one of the univalents dividing.

FIG. 60.—*R. subserrulata* 6072, tetraploid; first telophase with two groups of seven chromosomes.

FIG. 61.—*R. subserrulata* 6072, tetraploid; first telophase with fourteen chromosomes.

FIG. 62.—*R. rудiuscula* 3501, tetraploid; meiosis in megasporocyte, sister nuclei showing fourteen chromosomes, one in interkinesis and other entering second metaphase.

FIG. 63.—*R. acicularis* var. *sayiana* 3754, hexaploid; early diakinesis, twenty-one pairs, four groups of two pairs.

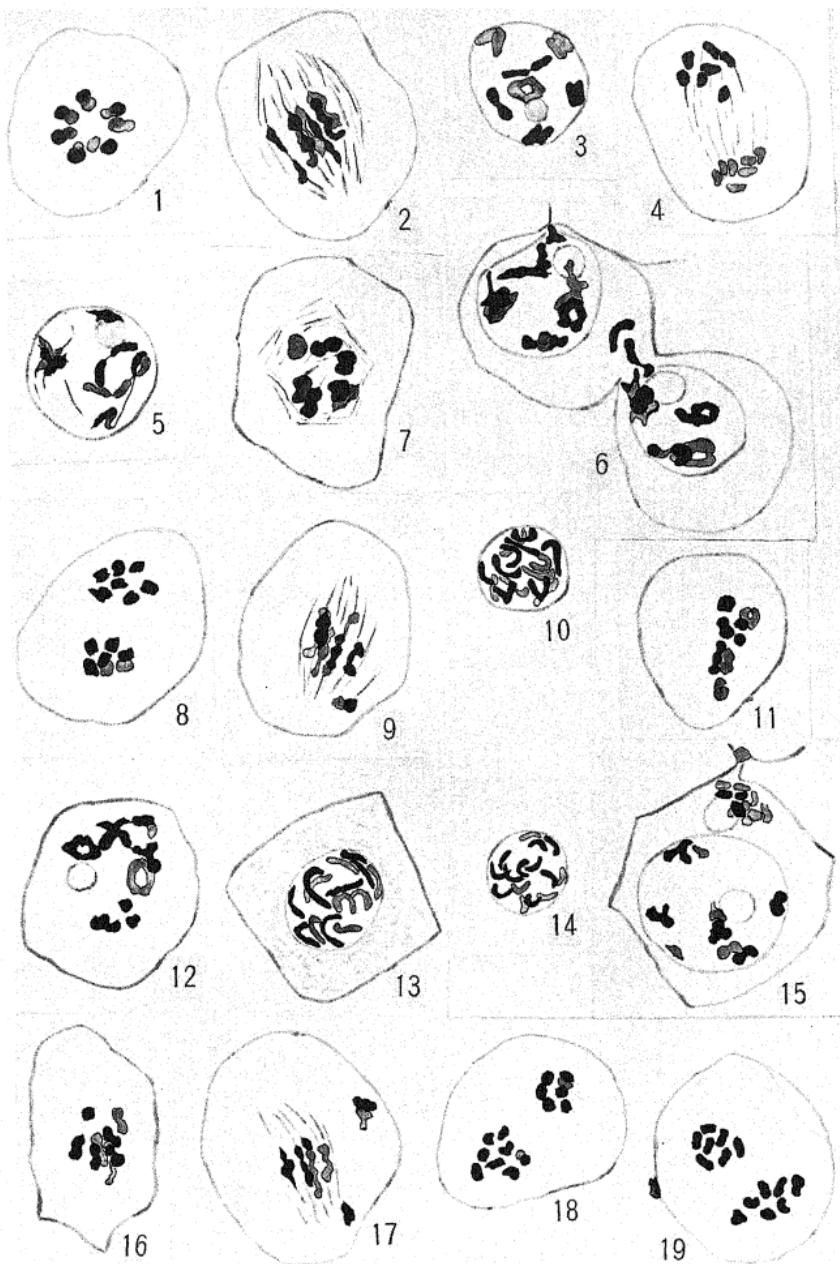
FIG. 64.—*R. underwoodii* 8270, hexaploid; early diakinesis with sixteen pairs in main and five pairs in subsidiary nucleus.

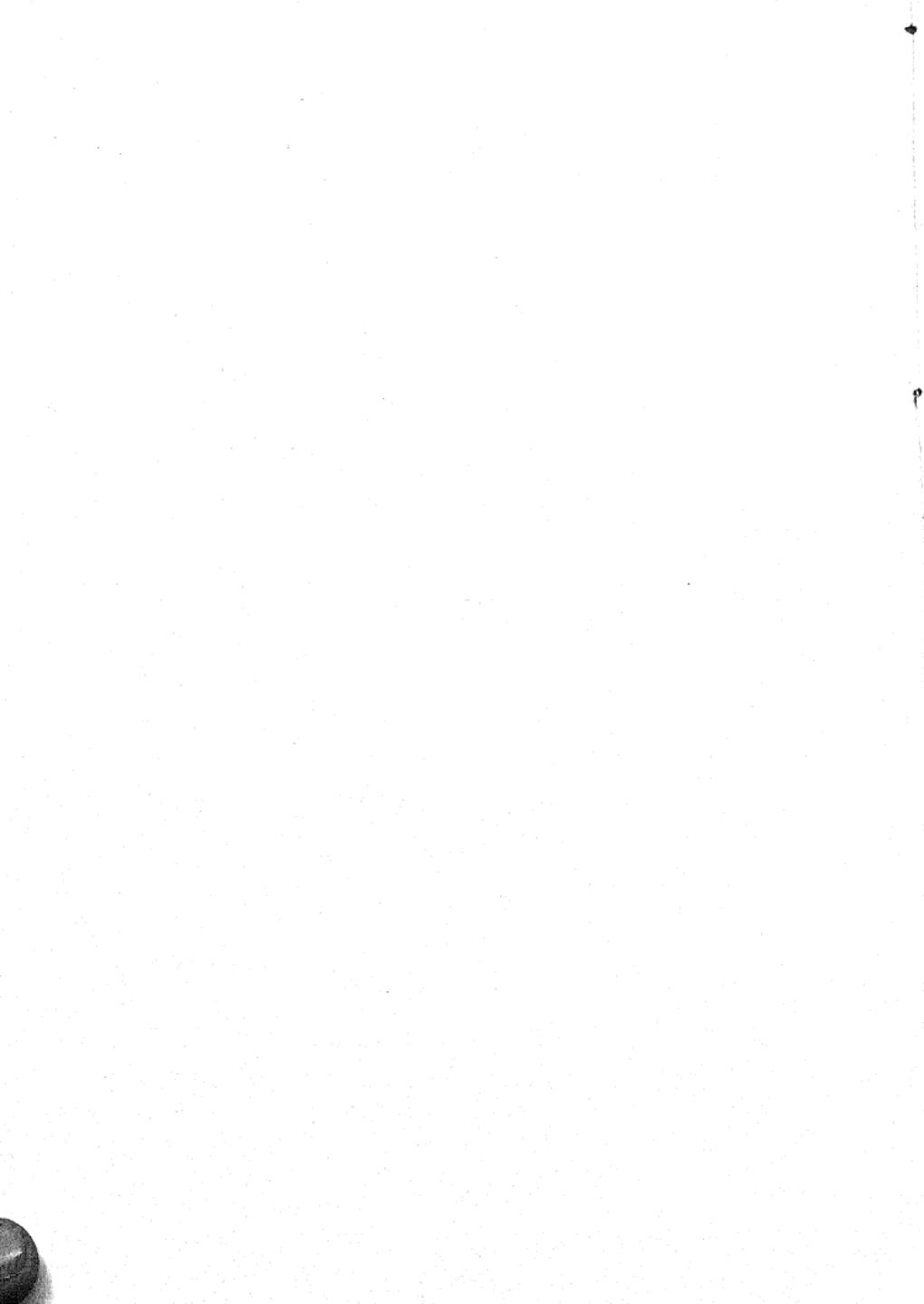
FIG. 65.—*R. spaldingii* E10, hexaploid; first metaphase plate with twenty-one pairs.

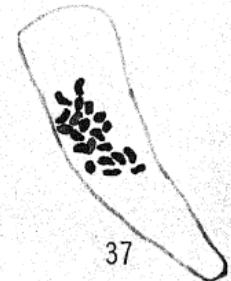
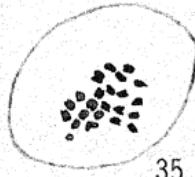
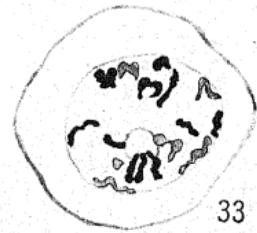
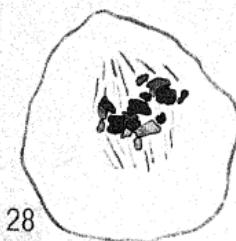
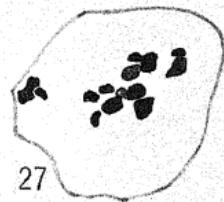
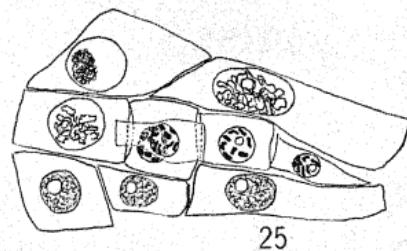
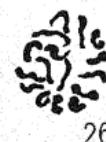
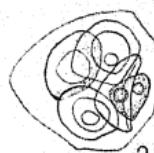
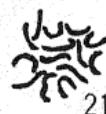
FIG. 66.—*R. macdougali* E8, hexaploid; five nuclei produced from one microsporocyte.

FIG. 67.—*R. acicularis* 6447, octoploid; diakinesis with twenty-eight pairs (ocular no. 20 and 1.8 mm. objective).

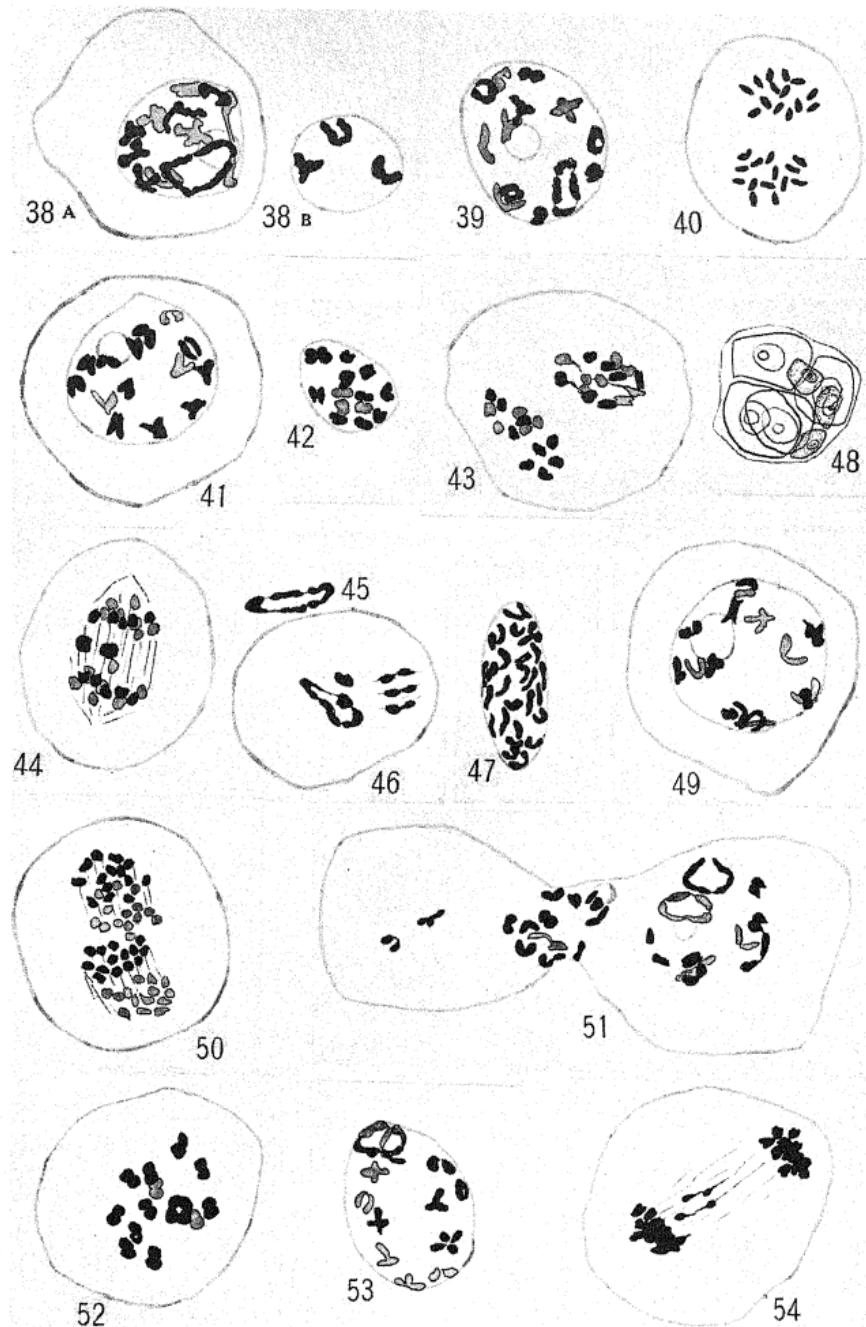
FIG. 68.—*R. acicularis* 6447, octoploid; first metaphase plate with twenty-eight pairs.

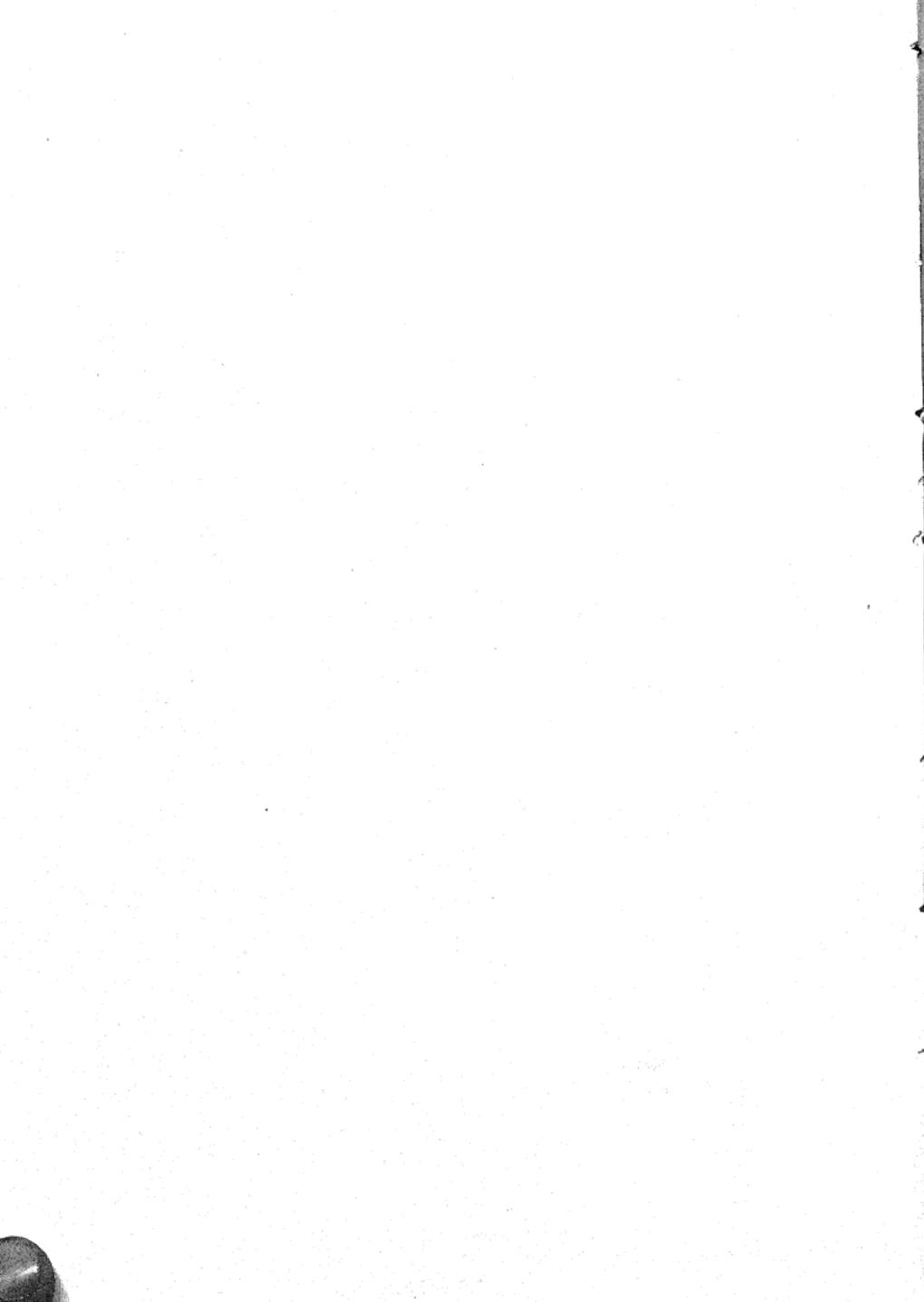


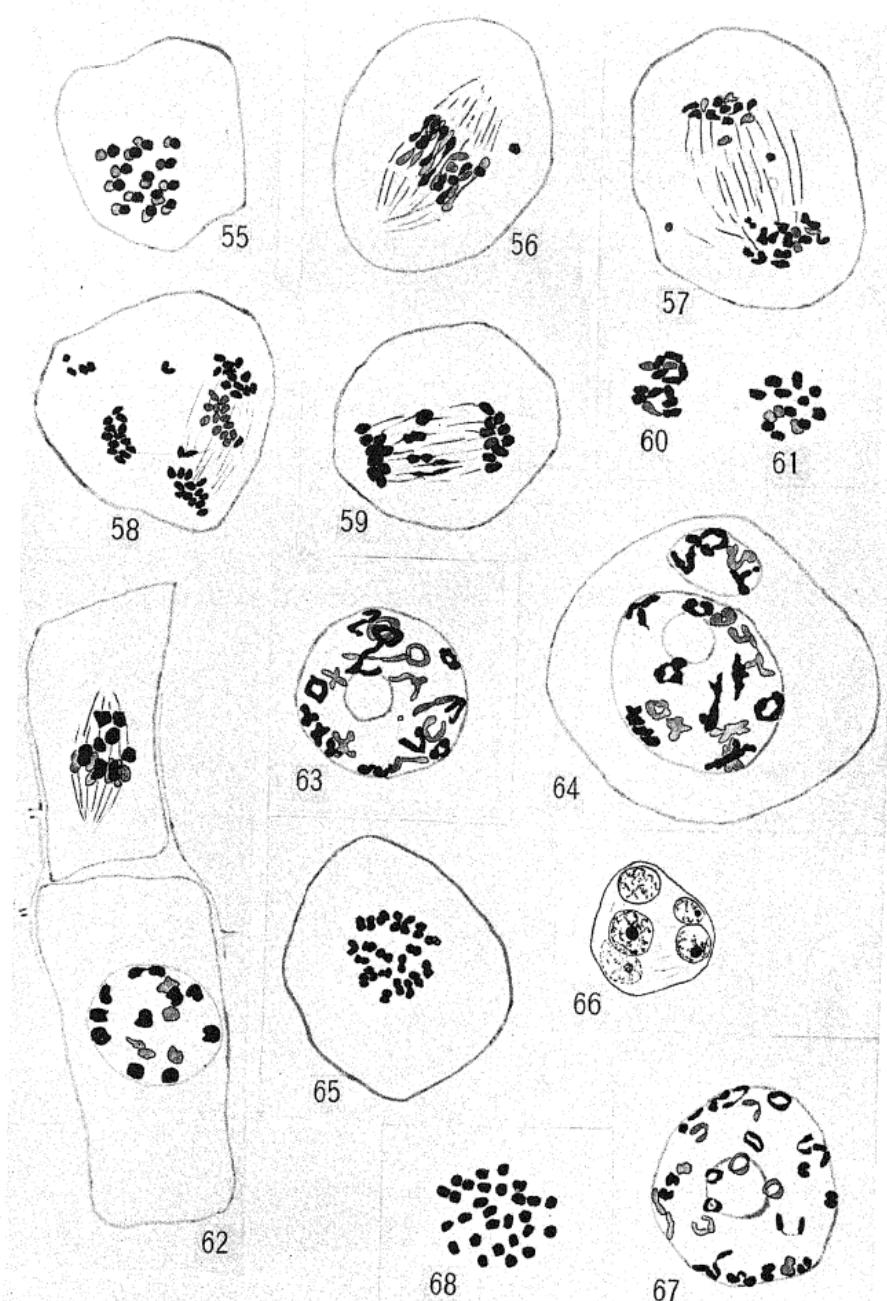


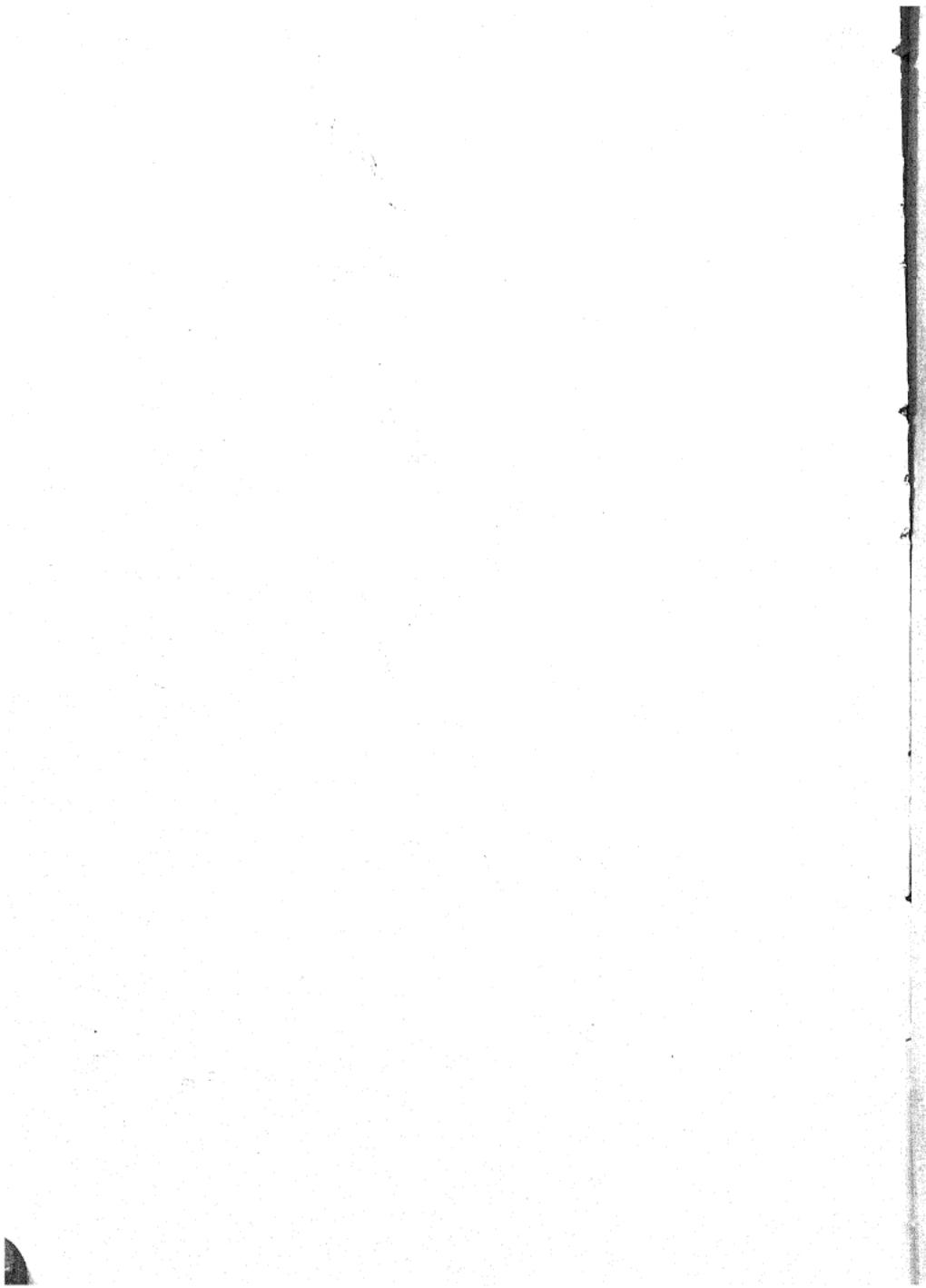












DEVELOPMENT OF DIONAEA MUSCIPULA

I. FLOWER AND SEED¹

CORNELIA MARSCHALL SMITH

(WITH PLATES XX-XXIV AND THREE FIGURES)

Introduction

This paper presents the results of a somewhat detailed study of the flower and seed development of the sole known species of *Dionaea*. A great number of researches have been published dealing with this extremely interesting insectivorous plant, since its original description in a letter of September 23, 1768, to LINNAEUS by JOHN ELLIS (17), but not one of these papers embodies an adequate study of its flower and seed development, and of the germination of the seed.

Dionaea was introduced into Kew Garden by WILLIAM YOUNG (15) in 1768. From that time forward botanists have been interested in the plant, primarily, however, in the movement and digestive powers of the leaf. EDWARDS (15) called attention to the fact that "the small spines, mentioned and figured by ELLIS, are the only irritable points, and that any other part of the leaf may be touched with impunity." CURTIS (9) and CANBY (5) made some of the earliest recorded observations on the insectivorous habits of *Dionaea*, as it grew in its native habitat near Wilmington, North Carolina. Although OUDEMANS (30) examined the leaves histologically, and noted the absence of nyctitropic movements, his report was chiefly concerned with observations on the sensitivity of the leaves.

Between 1874 and 1877 numerous papers on this plant were published. SANDERSON (36) demonstrated the similarity between the contraction of a leaf of *Dionaea* and that of the muscle of an animal in response to electrical stimulation. In 1875, DARWIN (11) not only gave a brief description of the roots and the enlargement of the stem from which the roots spring, and a detailed description of the leaves, but also reported the results of many interesting ex-

¹ Botanical contribution from the Johns Hopkins University, no. 100.

periments which he performed on the latter. Further, he concluded that the closure of the leaf is due to the contraction of a layer of cells on its upper surface. BALFOUR (1) discussed irritability, contraction, secretion, digestion, and absorption in *Dionaea*. He suggested that the spiral thickenings and their elasticity might play an important rôle in the nature and cause of contraction. It was upon the investigations of the anatomy and physiology of the leaf made by KURTZ (26), that MUNK (28) based his work. Although the major portion of the latter's research is concerned with an attempt to explain the action of the leaf from an electrical standpoint, he points out the additional fact that the sensitive hair is divided into two parts, a lever and a base, and that the base alone is capable of receiving stimuli. The results obtained and reported by DARWIN from his experiments on the leaves of *Dionaea* served as an impetus for the anatomical investigations of the vegetative organs of the entire plant by FRAUSTADT (18). Two other papers dealing with the mechanism which produces the movement of the leaves of *Dionaea* were published during this period respectively by DE CANDOLLE (6) and BATALIN (2).

Both GOEBEL (19) and MACFARLANE (27) described and figured the sensitive hairs of *Dionaea*, but the most recent, as well as the most detailed, description of them is that by HABERLANDT (22). Another paper of interest is that of DEAN (12), who, after studying *Dionaea* under native conditions, suggested that it would be more appropriate to call the plant "ant or beetle catcher" than to call it "fly-trap," because of its evident "specialization for the capture of ground insects." That the mechanism which causes the movement of the leaves has proved perplexing, is evidenced by the number of researches that have been and are still being conducted on the subject. The most recent papers offering an explanation of the movement of the leaves have been published by BROWN and SHARP (3), BROWN (4), and GUTTENBERG (21).

Dionaea can be propagated both by seed and by cuttings of the rootstock. HOLM (25) figures and briefly describes the germination of the seed. Although KURTZ states that it had long been a common practice among gardeners to propagate *Dionaea* by means of cuttings made from the inflorescence or flower stalk before the flowers

open, it was not until 1892 that this type of vegetative propagation was described and figured (HARSHBERGER 23, 24).

This brief summary includes the more important papers which have dealt with *Dionaea*. Several others, primarily of taxonomic interest, will be referred to later.

Habitat

Dionaea is a monotypic genus, indigenous, as reported by DARBY (10), CHAPMAN (7), and SMALL (37), to the wet sandy savannahs of eastern North Carolina and eastern South Carolina. The herbaria of the United States National Herbarium, the Missouri Botanical Garden, the New York Botanical Garden, Field Museum of Natural History, the Academy of Natural Sciences of Philadelphia, and the Gray Herbarium include specimens from the following localities in North Carolina: Wilmington, Burgaw, Lake Catherine, Bladen County, Bolton, Scott's Hill, Fayetteville, Dixon, and Hallsboro; and from Georgetown, South Carolina. Professor COKER informs me that he is publishing (8) a map which gives the distribution of *Dionaea*.

The writer visited eastern North and South Carolina the second week in April, 1928, for the purpose of seeing and collecting *Dionaea* in its native habitat. Plants were found growing near Wilmington in an open space in the pines, associated with *Drosera rotundifolia*, *Pinguicula lutea*, *P. elatior*, *Iris verna*, *Sarracenia purpurea*, and *S. flava*. At numerous other habitats from which *Dionaea* had been previously collected by various persons no plants were found, due no doubt to the late spring.

Material and methods

The plants of *Dionaea* used in this study were collected from one of its habitats near Wilmington, North Carolina. The material was shipped in living condition, and then either at once or after being established for some weeks in a greenhouse was fixed in a solution containing 2.5 cc. of glacial acetic acid and 6 cc. of commercial formalin to 95 cc. of 50 per cent alcohol. After dehydration it was imbedded by the usual paraffin method, cut in 5-10 μ serial sections, and stained on the slide. The stain used for the greater part of the work was Haidenhain's iron-haematoxylin. Flemming's triple stain

of safranin, orange G, gentian violet, and the double stain of safranin and light green were sometimes employed, however.

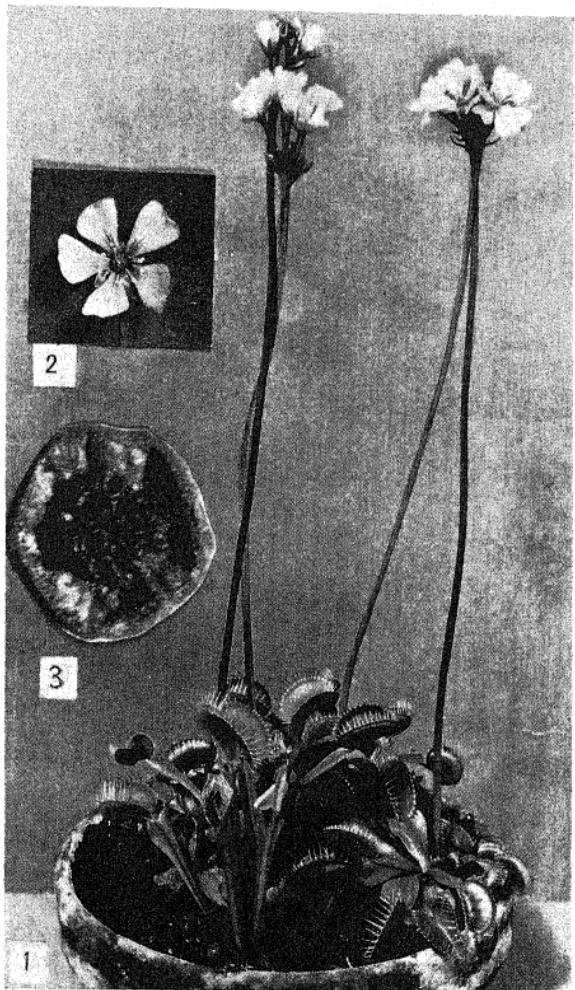
DEVELOPMENT OF INFLORESCENCE

In the early spring, the apex of the sympodial, perennial rhizome of *Dionaea* sends up successive aerial leaves. When from eight to twelve leaves have been developed, a leafless floral axis begins to push out of the terminal bud of the short, horizontal rhizome. Plants collected in North Carolina on April 15, 1927, and examined in Baltimore on April 18 showed scapes 2-3 cm. long. Plants collected April 8, 1928 in the field 2 miles from Wilmington just off the New Bern road possessed floral axes 1-2 cm. in length. When the scape is mature, it may reach 20 cm. or more in height. This axis of the mature flower stalk or scape is about 4 mm. in diameter, and bears a cyme of from two or three up to twelve or fourteen flowers (fig. 1). Not more than four or five flowers of a cyme are open at one time. The flower lasts for about three days.

Each flower has five small green, elliptical, persistent sepals pointed at the apex; five white, cuneate petals notched at the apex; eleven to fifteen stamens (usually fifteen); and a single, compound pistil composed of five carpels (fig. 2). This radially symmetrical, pentacyclic flower develops essentially in the normal manner, the five members of each perianth cycle being synchronous in origin and the different cycles appearing in the usual sequence.

STAMENS AND POLLEN GRAINS

The third and fourth cycles of organs to be differentiated in the flower are composed of stamens. Members of the last formed, epipetalous cycle are often doubled (fig. 6A, B). PAYER (33) was the first to point out that the stamens of *Dionaea* develop in two whorls. He states, however, that the alternipetalous stamens appear interior to and before the epipetalous stamens; and with regard to the number of stamens developed in each whorl he says, "Rarement chacun de ces verticilles n'est composé que de cinq étamines; le plus souvent on en compte plus de cinq, parce que à la place d'une il s'en développe deux." Moreover, in fig. 19, pl. 38, he shows a young flower in which the alternipetalous stamens are doubled, whereas in fig. 20, pl. 38, he shows a slightly older flower in which the epipetalous



FIGS. 1-3.—Fig. 1, plants showing different types of leaves, mature scapes, and cymose inflorescence (note wasp captured by leaf of plant on right); reduced one-half; fig. 2, flower with 5 sepals, 5 petals, 14 stamens, and pistil composed of 5 carpels; slightly reduced; fig. 3, receptacle of mature fruit with cup-shaped bolsters of placental tissue (photographed by A. F. SKUTCH); $\times 15$.

stamens are double. Later, in describing the stamens of *Dionaea*, EICHLER (16) says, "Nach PAYER gehören dieselben 2 ursprünglich 5 zähligen Kreisen an, von denen der alternipetale zuerst entsteht; bei Ueberzahl findet Dédoublement statt, *namentlich im epipetalen Quirl*." (fig. 88 C)." In this figure (p. 224) EICHLER has indicated the "dédoublement" of the epipetalous stamens which he accredits to PAYER. An examination of PAYER's description and figures, however, makes it clear that he believed that either the alternipetalous or the epipetalous stamens could undergo "dédoublement." Although the portion of EICHLER's statement which I have italicized agrees with the facts, it has been wrongly attributed to PAYER. DIELS (13), RENDLE (34), and others cite PAYER for this explanation, which, however, is not found in PAYER but in EICHLER. Further proof of EICHLER's figure may be had by following from their source the course of the vascular bundles of the stamens of young flowers. If this is done, it is found that when the flower has more than ten stamens, one or more of the five vascular strands which supply the five epipetalous stamens are divided, and that two epipetalous stamens have developed in place of one where the strand is divided. Fig. 6A shows a cross-section of the receptacle of a young flower containing fourteen stamens, in which five vascular strands respectively of the outer alternipetalous and of the inner epipetalous stamens are visible. Fig. 6B shows a similar section of the same flower 60 μ nearer the apex, in which four of the five vascular strands of the epipetalous stamens are divided. If, on the other hand, the flower develops but ten stamens, the five outer alternipetalous stamens alternate with the five inner epipetalous stamens, as is shown in fig. 5C. Although most of the taxonomists report ten to twenty stamens for *Dionaea*, none of the flowers which I have as yet examined possessed more than fifteen. I have been unable, therefore, to determine what the arrangement of the stamens would be in a flower developing more than fifteen stamens.

Each stamen originates as a protuberance involving dermatogen and periblem. As the protuberance grows it pushes upward, and gradually broadens above the slender base (figs. 5, 7, 9). The upper enlarged end is the anther, in which four microsporangia develop; the slender base becomes the filament.

¹ Italics mine.

The microsporangia develop in the usual manner. A cross-section of a young anther shows usually a single primary archesporial cell in each of the four corners immediately beneath the epidermis. In the upper right corner of fig. 8 are shown two primary archesporial cells, a relatively rare case. A longitudinal section shows that, in reality, each cell seen in transverse section is one of a row of from five to seven cells extending the full length of the anther. Each cell of this row divides into an outer parietal and an inner sporogenous cell. By radial and pericinal divisions, the primary parietal cells then give rise to from three to five concentric layers of wall cells (fig. 10).

Subsequent differentiation among the wall cells results in the formation of the tapetum, the "middle layers," the endothecium, and the epidermis. In following the further history of each layer, changes are seen to occur first in the innermost layer of wall cells, the tapetum. Its cells enlarge, become granular, and often binucleate (figs. 16, 21). In enlarging, the tapetal cells encroach upon the "middle layer," crush, and finally absorb the one or two rows of cells of which the middle layer is composed (figs. 21, 23). Next the cells of the endothecium begin to increase in size. A transverse section of a microsporangium (fig. 23) made at this stage shows the cells of the endothecium to be approximately as large as those of the epidermis, and the tapetum greatly reduced as it is being gradually absorbed in the maturing of the spores. Finally the tapetum completely disappears, the walls of the endothecium become prominently thickened in bands, and the cells of the epidermis disintegrate (fig. 25). This same figure shows the weak place in the sporangium wall, where the slit, by means of which the pollen grains may escape, is to be formed.

By successive divisions of the primary sporogenous cells forty-eight to sixty microspore mother cells are formed. These mother cells have increased, by the time of synizesis, from a diameter of $15-17 \mu$ to one of $25-30 \mu$. Concurrently the diameters of their nuclei enlarge from a diameter of 10 or 11μ to one of 15 or 17μ . The nucleoli do not change in size, remaining about 4μ in cross-section. Due to the fact that the nucleus of the spore mother cell increases in size as the cell matures in preparation for meiosis, however, the

nucleolus is proportionately larger in the early stages. In a single microsporangium all nuclear changes occur almost simultaneously (fig. 16), but the four sporangia of the same stamen do not always show the same stage of development.

The nuclear phenomena which occur in the spore mother cells preparatory to and during the formation of tetrads may be described as follows. Small chromatin granules or prochromosomes, similar to those described by ROSENBERG (35) in *Drosera* and by OVERTON (31) in *Thalictrum*, *Calycanthus*, and *Richardia*, are visible in the resting nucleus. They are arranged in pairs of units lying side by side on the linin threads, and are distributed about the periphery of the nucleus, close to the nuclear wall (fig. 11). As the nucleus enters the prophase (fig. 12), the chromatin threads become more distinct and then pass into the synizesis contraction (fig. 13). The paired chromatin threads become massed together at one side of the nuclear cavity in synapsis. Usually the nucleolus lies adjacent to but distinct from the chromatin mass. In some cases, however, it is surrounded by filaments of chromatin.

When the threads of the synaptic knot loosen up, the loops of the spireme formed appear to be double, with paired chromatin granules scattered along them (fig. 14). The spireme then segments into fifteen pairs of bivalent chromosomes, which are found scattered throughout the nucleus (fig. 15). Some parts of the chromosomes stain more deeply than other parts, as is shown in fig. 15. As a consequence of gradual shortening and thickening, the chromosomes acquire a compact form and draw closer together (fig. 17). The nuclear membrane is visible about the chromosomes in diakinesis. Fig. 16 shows a longitudinal section of a microsporangium in which the cells are rounded up and more or less separated from one another (segments of the original walls being still visible between some of the cells), and in which the nuclei of all the microsporocytes are in diakinesis.

The microsporocytes of a sporangium adjacent to the one shown in fig. 16 have advanced a stage further; the spindle is formed for the first meiotic division. Fig. 18 shows the V-shaped chromosome tetrads as they appear in metaphase with the spindle fibers attached. The double character of the chromosomes, as they move apart in the anaphase, becomes clearly discernible (fig. 19). Four nuclei, each

having fifteen haploid chromosomes, result from the second division, which occurs immediately after the first (fig. 20).

Walls appear between the four nuclei (fig. 21), thus cutting out the four microspores of a tetrad. As the microspores mature each develops an outer spore coat, the exine, containing six to eight germ pores, and an inner spore coat, the intine (fig. 23). The exine is composed of an outer layer, made up of slender prismatic elements standing perpendicular to the surface, interspersed among which are longer, pointed prisms or spines; and of an inner layer of apparently homogeneous structure (fig. 26). The two layers when fully developed are of about equal thickness (each 2.5μ). Because of the prominence of these two layers of the exine in the mature spore, they were at first mistaken for the exine and intine respectively, but on closer examination it was seen that they together make up the exine, and that the intine is a separate, much more delicate coat adjacent to and completely surrounding the protoplast. The intine is very plastic; it becomes more easily distinguishable when it grows out through the germ pores of the exine to form the pollen tube (figs. 25, 26).

In fig. 23 each microspore or very young male gametophyte of the tetrad is shown with its two spore coats incasing a resting nucleus surrounded by vacuolated cytoplasm. This nucleus divides into the generative nucleus and tube nucleus (fig. 25). This is the usual condition of the microspores when shed. In some instances the generative nucleus has already divided, however, forming the two male nuclei. Fig. 26 shows a section through a tetrad of germinating pollen grains as found on the stigma. In each grain the pollen tube protrudes from a germ pore; in two of the pollen tubes a tube nucleus is visible; the male nuclei have not as yet passed into the pollen tube. In the interior of the style can also be seen portions of pollen tubes, identified by their tubular structure and by their reaction to stain. The cell contents of the latter could not clearly be distinguished.

CARPEL AND SEED

The last cycle of members to be formed in the flower is one of five carpels, which, like the stamens, originate as outgrowth from the periblem involving also the dermatogen. The prominence which

gives rise to the carpels is at first convex, then flat (fig. 5), and later concave (fig. 7). The concave structure is divided into five carpels, each of which in turn is differentiated into an upper and a lower region. As the upper portions elongate they curve inward, come into contact with each other, and later coalesce to form a column, known as the style, containing a central stylar canal (figs. 9, 27, 32). The end of the style is divided into numerous projections or papillae, composed of cells with large nuclei and dense cytoplasm which later serve as receptive structures for pollen.

In the young flower the papillae curve inward, forming a small knoblike structure, the stigma (fig. 52A). When the style has reached a length of about 2.5 mm., these papillose bifurcations of the stigma straighten up, bend outward, and expose the receptive tissue. Any pollen which at this time comes in contact with the stigma may germinate. Fig. 52B shows a pollen tetrad on the stigma. Later the papillae of the stigma shrivel and coil outward. They are found in this condition surmounting the dried-up style of the mature fruit.

The wall of the ovary is formed by the coalescing of the lower portions of the five carpels. The term "Parakarpe Gynäceen" has been applied by GOEBEL (20) to the ovary of *Dionaea*, because its carpels are joined by their margins only. As it grows, the cavity in which the anatrapous ovules are to develop gradually enlarges (figs. 9, 32, 52A). From the floor of the cavity of the very young ovary, numerous erect hypodermal protuberances appear in serial succession, from the inside outward, around the growing point of the flower, as illustrated and described by GOEBEL (20) and as shown in fig. 9. Each protuberance becomes differentiated into a basal stalklike structure, the funiculus, and terminal region, the nucellur or ovule. When the protrusion is about five cells across in transverse section and twenty-five in longitudinal section, the nucellar end becomes slightly curved; and, concurrently the first integument is initiated about its base (fig. 27). External to the first integument a second forms, while the nucellus curves progressively downward beside the funiculus. A longitudinal section of an ovule made at a slightly later stage (figs. 32, 33) shows the ring of tissue of the first integument surrounding all except the apex of the nucellus; and the

second integument, part of which is formed from the funiculus, encircling the first for about two-thirds of its length. The integuments continue to elongate and soon overtop the nucellus, the first integument drawing successively closer together over the nucellus, leaving only a narrow passage way, the micropyle.

Each integument is composed of two or three layers of cells. The layers of the inner integument, except where they overtop the nucellus, consist of thin-walled parenchyma cells which remain unspecialized. In the chazal end of the ovule, however, some of the thin-walled cells have become disorganized and replaced by air spaces (fig. 63). On the other hand, those cells which overtop the nucellus finally become heavily lignified and form an inner seed cover or lid (figs. 70, 71), which is pushed off in the course of the germination of the seed. This structure resembles that which NETOLITSKY (29) has described in *Drosera rotundifolia*.

Only the outermost layer of cells of the outer integument shows any specialization, the inner layers, with the exception of a group of thickened cells investing the vascular strand, remaining unmodified and difficult to distinguish from the cells of the inner integument. The former develops into an outer protective layer, made up of closely packed, radially arranged, heavy-walled, columnar cells (fig. 63). This might be called the palisade layer, because its cells resemble in form the palisade cells of a leaf; or the cells of which it is composed could be called Malpighian cells, except that the "luminous line" usually present in the latter is wanting in the seed of *Dionaea*. The external walls of the cells become progressively and evenly thickened, whereas the radial walls first become thickened in strips (fig. 63) and later in coalesced bands (fig. 71).

If the mature fruit is examined, it is seen to contain numerous seeds grouped on a basal placenta. EDWARDS (15) describes the seeds and their insertion as follows: "Seeds black, shining, obovate, very acute at the lower end, half buried in the cavities of the honey-combed receptacle." DRUDE (14) says in regard to them: "Samen zahlreich auf der gewölbten Blütenachse central eingefügt." The writer's observations confirm these descriptions, except she finds that the lower end of each seed, even when mature, is only slightly imbedded in a cup-shaped bolster of receptacular tissue. The exact

shape and arrangement of these collars of receptacular tissue are shown in the photograph of a mature floral receptacle (fig. 3). In a longitudinal section of a much younger ovary (fig. 52A) this tissue is seen projecting up about the micropylar end of each ovule.

The single bundle or vascular strand of each ovule develops in the funiculus, which, as has been stated, becomes fused with a part of the outer integument. The strand extends to the chalazal region of the ovule; it shows no tendency to turn or twist in its course. In the young ovule the first vascular tissue to be developed consists of narrow, elongated, densely protoplasmic cells, the procambium (fig. 33). It is from these cells that the xylem, consisting of long, slender, spirally thickened cells, and the phloem parenchyma of the mature strand develop. This mature strand, which is surrounded by a group of thickened cells of the outer integument, measures about 55μ in transverse section.

The nucellar tissue within the integuments shows some interesting differentiations. The epidermal cells, except those immediately above the sporogenous cells, form a layer of radially placed, closely packed, thin-walled cells which enlarge immensely and in so doing become more and more conspicuous (figs. 33, 46, 58). They increase from 7.5 to 62.5μ in anticlinal length from the time that the spore mother cell is formed (fig. 33) to the time of fertilization (fig. 58). These radially elongated cells completely surround a central strand, four to five cells wide, which extends from the prothallial cells to the vascular strand and is composed of longitudinally elongated cells (figs. 46, 58). Since the latter lie in direct contact with the vascular strand, at the chalazal end of the ovule, it seems reasonable to assume that the prothallial cells receive their nourishment through them.

This enlargement of the nucellar cells resembles that described in *Drosera rotundifolia* by Miss PACE (32), who states further that these cells, together with the air spaces developed in the chalazal region of the ovule, greatly reduce the specific gravity of the seed. The latter statement is hardly true with respect to the enlarged nucellar cells of *Dionaea*, because of the fate of the cells and of the spaces occupied by them. As the embryo develops in *Dionaea* the endosperm encroaches upon the nucellar tissue (fig. 58), disintegrat-

ing and absorbing both the central strand of elongated cells and the encircling radially-attenuated cells (fig. 63). Finally, in the mature seed the cellular endosperm completely fills the space occupied by the nucellus, and also the space occupied by the thin-walled cells of the integuments (fig. 71). As a result the specific gravity of the seed does not decrease but is actually increased.

Since the latest stage of seed development of *Drosera* figured by Miss PACE shows fertilization, it seems reasonable to suppose that she did not follow out the subsequent development of the endosperm and the fate of the nucellar tissue; hence her conclusion that the enlargement of the nucellar cells reduces the specific gravity of the seed is not valid. Further, an examination of a series of slides of *Drosera rotundifolia* made by C. A. PETERS at Ann Arbor, Michigan, and now in the collection of slides of the Johns Hopkins University, shows that the endosperm develops at the expense of the nucellar tissue very much as it does in *Dionaea*.

The single archesporial cell of the megasporangium is set off immediately beneath the epidermis of the nucellus, about the same time that the first integument appears. This cell is easily recognized by its large size and dense protoplasm, as well as by its apical position in the nucellus (fig. 28). It is about 17μ in transverse section, and contains a nucleus which has a diameter of 10μ . By mitotic division (fig. 29) it gives rise to the primary sporogenous cell and primary parietal cell (figs. 30, 31). The latter is concerned with the production of a small portion of the megasporangium wall, but not in the formation of distinct layers of wall cells as is true of the parietal cells of the microsporangium.

The first division of the primary parietal cell is by a radial wall, which is easily discerned when it is perpendicular to the plane of the section (figs. 34, 35). Although it is impossible to follow the subsequent divisions of the individual parietal cells, as they are no longer distinguishable from the adjoining nucellar cells, it is evident that by periclinial divisions two or at most three layers of cells are formed above the sporogenous cells (figs. 44, 48). These layers are at once infringed upon and absorbed by the developing megasporule (figs. 46, 51).

The primary sporogenous cell usually develops directly into a

single megasporangium mother cell (figs. 33, 34); rarely there are two (fig. 35). Since no further development of the two mother cells was observed in the several hundred sections studied, it is hardly probable that these ever progress far enough to develop two embryo sacs or embryos in an ovule. The single mother cell, on the other hand, increases in size and passes through nuclear changes similar to those described in the microspore mother cells. Paired prochromosomes, of approximately the same number as the definitive chromosomes, are visible in the resting nucleus. In the late prophase the chromatin threads become more distinct and then pass into the synaptic contraction. Fig. 36 shows the loosening up of the spireme and the paired chromatin centers about which the chromosomes develop. The spireme is then segmented into fifteen pairs of bivalent chromosomes, each of which becomes more distinct as diakinesis approaches (fig. 37). The formation of the spindle and further nuclear phenomena in the division of the megasporangium mother cell were not observed. In fig. 47, however, it is evident that the reduced number of chromosomes is present at the second division of the functional megasporangium; in other words, there are fifteen haploid chromosomes.

The mother cell divides to form two (fig. 38), then by a second division of one of the daughter cells it forms three (fig. 39); or in other cases both daughter cells divide and four potential megasporangia are formed (figs. 41, 42). These four spores are usually arranged in an axial row. That the two daughter cells do not necessarily divide simultaneously to form the tetrad of megasporangia is shown by figs. 39 and 40. Three of the four potential megasporangia degenerate, leaving but one functional. Usually in *Dionaea*, as in other angiosperms, the non-functional cells of the micropylar end of the ovule disappear (figs. 44, 48, 49). Fig. 43 shows a single active megasporangium with the degenerating cells in the chalazal end of the ovule, an uncommon occurrence. The last vestige of the degenerating megasporangia disappears before the eight-nucleate embryo sac is completely organized.

EMBRYO SAC, ENDOSPERM, AND EMBRYO

The embryo sac is initiated with the division of the functional megasporangium, after which the daughter nuclei move apart, one going to each end of the embryo sac. The cytoplasm between the nuclei

becomes more and more vacuolated (figs. 44, 45). Subsequent divisions take place rapidly and simultaneously. Fig. 48 shows the four nuclei and fig. 50 the eight nuclei resulting respectively from the second and third divisions; and fig. 49 shows that the third division occurs at the same time in all four of the nuclei resulting from the second one.

One of the four nuclei at each end of the embryo sac, the polar nucleus, migrates toward the center. The three nuclei remaining at the micropylar end form the egg apparatus; the three nuclei remaining in the chalazal end form the antipodal cells (fig. 51). The egg apparatus is quite normal, consisting of two synergids and an egg. Each of the former has a notch, is capped by a filiform apparatus, and contains one or more vacuoles in its lower end (fig. 51). The egg cell has a larger nucleus than the synergids, and usually lies nearer the center of the embryo sac, below the synergids. The two polar nuclei, which come to rest in the middle of the sac just below the egg, are of about the same size, each containing a large nucleolus. The two nuclei may lie side by side at the same level, or one may be nearer the egg and the other nearer the antipodal. They are always surrounded by a mass of cytoplasm, some strands of which extend upward to connect with the layers of cytoplasm that surround the lower end of the egg, while other strands reach to the notches in the synergids. The antipodal cells which are located in the chalazal end of the sac never become conspicuous; they often disappear shortly after they are formed.

Actual fusion of the egg and sperm cells was not observed, although hundreds of sections, made at about the time of fertilization, were examined. Fig. 51 shows the polar nuclei in contact with each other, and fig. 53 shows the polar nuclei in the process of fusion, thus forming the primary endosperm nucleus. In some cases the polar nuclei fuse before, and in other instances after fertilization. Fig. 54 shows the primary endosperm nucleus with its characteristically large nucleolus. The mean diameters of the primary endosperm nucleus and nucleolus are 18 and 8 μ respectively, ascertained by averaging twenty measurements. Referring again to fig. 54, the fertilized egg can be seen below the synergids, the contents of which are entirely obscured by a darkly staining substance, that was probably

introduced with the entrance of the pollen tube. That the synergids do not disappear immediately after fertilization is evidenced by the fact that remains of them and their degenerating nuclei are visible after several divisions of the fertilized egg (figs. 59, 61).

The primary endosperm nucleus divides before the fertilized egg, and its subsequent divisions occur more rapidly than those of the egg. The nuclei resulting from the first and second divisions of the endosperm nucleus are shown in figs. 55 and 57; they are elongated and each contains two or more nucleoli. The egg has not yet divided. The early mitoses occur simultaneously in all nuclei (fig. 56). Later, however, when the endosperm has become multinucleate, the nuclei in one region show a different stage of development from those in other regions of the endosperm (fig. 63).

With the multiplication of the nuclei in the endosperm, the cytoplasm surrounding the former becomes more and more vacuolated; and, as a result of the union of numerous small vacuoles, a single sap-filled central vacuole develops. With this increasing vacuolation the nuclei are caused to assume a peripheral position, a group of them becoming arranged in a funnel-shaped mass near the chalazal end of the embryo sac. From fig. 58 it will be seen that, although the fertilized egg has not divided nor have the synergids completely disintegrated, the endosperm has progressed to the condition just described, the lower funnel-shaped portion of it having already begun its inroads upon the surrounding nucellar tissue. As growth continues this lower end of the endosperm, which acts somewhat as a haustorium, progressively encroaches upon and absorbs the nucellar cells, until it finally comes in direct contact with the vascular strand at the chalaza. A median longitudinal section through the ovule (fig. 63) shows a relatively thin layer of cytoplasm, with free nuclei surrounding a central vacuole and extending from the embryo to the vascular strand. A mass of cytoplasm and nuclei are to be seen in contact with this strand. A surface view of the endosperm, made at the same stage (fig. 64), shows the nuclei about equally spaced in a uniformly distributed layer of cytoplasm.

When free nuclear division has ceased in the endosperm, walls are formed between the nuclei, as a result of which an outer layer of cells is developed about the central cavity (fig. 69). With the

initiation of wall formation, each nucleus of the hundred or more then present becomes surrounded by a somewhat denser mass of cytoplasm, strands of which extend to the encircling cytoplasm of the adjacent nuclei (figs. 65, 66). Each of the connecting strands becomes less dense and more curved as it passes outward from the mid-line between adjoining nuclei. STRASBURGER (38) has described and figured, in *Agrimonia eupatoria*, *Reseda odorata*, and certain other plants, a condition similar to this. The distribution of the cytoplasm about the nuclei at this stage makes it possible to predict that the cell walls will appear in a plane equidistant between neighboring nuclei and at right angles to the connecting strands. The next stage observed is shown in fig. 67. The fibrillae, which had their origin in the connecting strands of cytoplasm, show the equatorial thickenings which later split to form two parallel cell plates (fig. 68). A thin continuous cell wall is soon formed between the two cell plates and the fibrillae begin to disorganize. The cells thus formed now divide and gradually replace the central cavity (fig. 69). Finally the entire embryo sac, except the part occupied by the embryo, is filled with the cellular endosperm (figs. 70, 71).

In contrast to the free nuclear division of the primary endosperm nucleus, the first division of the nucleus of the fertilized egg is accompanied by the formation of a cell wall. This first division is transverse to the long axis of the embryo sac, moreover, and results in the formation of the suspensor and embryo (fig. 59). Of the two the suspensor cell is situated nearer the micropyle, is larger, and tapers toward the micropyle, while the embryo proper is hemispherical. Furthermore, the first division of the suspensor cell usually precedes that of the egg (fig. 60).

The first and second divisions of the embryo are longitudinal and transverse respectively. Fig. 61 shows the four cells resulting from these divisions. The cytoplasm of the cells of the embryo is much denser than that of the suspensor cells. The cells of the embryo continue to multiply, and soon an outer layer of cells, the dermatogen, is cut off by periclinal walls (fig. 70). In the meantime the suspensor cells have divided and formed a cylindrical structure composed of six to eight cells (fig. 70), the ultimate fate of which was not determined. The embryo in the mature seed consists solely of two small

cotyledons and a short radicle (fig. 71). The structure of the embryo becomes further differentiated in the process of germination. It will be more fully described in a second paper, on *Dionaea muscipula*, dealing specifically with the germination of the seed and seedling. The bearing of the facts here recorded on the relationships of *Dionaea* will also be deferred to the second paper for discussion.

Summary

1. The terminal bud of the short perennial rhizone of *Dionaea* forms a scape which bears a cymose inflorescence of from two to fourteen actinomorphic, pentacyclic flowers.
2. The arrangement of the vascular bundles of the stamens in the receptacle of young flowers is considered as further proof of PAYER's statement that the stamens develop respectively in alternipetalous and epipetalous whorls. Moreover, when more than ten stamens (five in each cycle) develop, the vascular bundle of one or more of the five epipetalous stamens is divided and supplies the additional stamen or stamens. This is not in accord with the view held by PAYER that "dédoulement" may occur in either whorl.
3. The four microsporangia of each stamen develop in the usual manner.
4. In the early prophase of the microsporocytes, of which forty-eight to sixty develop in each sporangium, prochromosomes are discernible.
5. Each of the four nuclei resulting from the second meiotic division of the microsporocyte has fifteen haploid chromosomes.
6. The mature pollen grains hang together in tetrads and each contains six to eight large germinating pores.
7. The "paracarpous" ovary of *Dionaea*, which is composed of five carpels, develops numerous anatropous ovules on a basal placenta, with the tip of each slightly imbedded in a cup-shaped bolster of placental or receptacular tissue.
8. The outermost layer of cells of the second integument forms an outer seed coat of closely packed, radially elongated, heavy-walled, columnar cells; while those cells of the inner integument which overtop the nucellus form a lid or cover which is pushed off when the seed germinates.

9. The single vascular strand which extends to the chalazal end of each ovule shows no tendency to turn or twist in its course.
10. The outer layer of nucellar cells, except where it overtops the prothallial cells, increases enormously in radial dimension, surrounding a central strand of longitudinally elongated cells.
11. By division of the single hypodermal archesporial cell, two or at most three layers of parietal cells are formed above the embryo sac.
12. Rarely more than a single megasporangium is developed; the mother cell divides to form two, then four potential megasporangia; the divisions of the daughter cells do not necessarily occur simultaneously.
13. Usually, as in other angiosperms, the three micropylar potential megasporangia degenerate and the chalazal one functions. In one instance the three basal spores degenerated and the micropylar one functioned.
14. The embryo sac resulting from three successive divisions of the functional megasporangium contains, when ready for fertilization, the usual egg apparatus, polar nuclei, and antipodal cells.
15. The primary endosperm nucleus usually completes several mitoses before the fertilized egg divides to form the embryo and suspensor.
16. As the endosperm increases it progressively encroaches upon the surrounding nucellar tissue, and by the time that free nuclear division has ceased in the former all nucellar tissue has disappeared.
17. The mature seed contains a small basal embryo, consisting of two cotyledons and a short radicle surrounded by the cellular endosperm.

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JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD.

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EXPLANATION OF PLATES XX-XXIV

All figures were drawn with the aid of the camera lucida, except figs. 1-3.

FIG. 4.—Transverse section of very young inflorescence; sepals differentiated in all flowers, petals in lowest flower on left only; $\times 38$.

FIG. 5.—Longitudinal section of young flower, showing protuberances giving rise to stamens and carpels; $\times 38$.

FIG. 6.—A, transverse section of receptacle of young flower containing 14 stamens, showing the 5 vascular strands respectively of outer alternipetalous and inner epipetalous stamens; B, similar section of same flower 60 μ nearer apex, in which 4 of the 5 vascular strands of epipetalous stamens are divided; C, similar section of flower developing only 10 stamens, 5 outer alternipetalous

alternating with the 5 inner epipetalous (*s*, sepal; *p*, petal; *a*, alternipetalous; *e*, epipetalous); all $\times 18$.

FIG. 7.—Longitudinal section of flower of about same age as those shown in preceding figure; stamens clavate, carpels concave; $\times 38$.

FIG. 8.—Transverse section of young anther, showing 2 primary archesporial cells in left corner, primary archesporial cell in mitosis in right corner, and primary parietal and sporogenous cells in each of lower corners; $\times 172$.

FIG. 9.—Longitudinal section of flower with stamens in spore mother cell stage and ovules appearing in base of ovary; $\times 38$.

FIG. 10.—Transverse section of single anther; microspore mother cells surrounded by 3 or 4 concentric layers of wall cells; $\times 172$.

FIG. 11.—Single microspore mother cell in early prophase, showing paired prochromosomes and nucleolus; $\times 925$.

FIG. 12.—Nucleus in late prophase; $\times 925$.

FIG. 13.—Nucleus in synapsis; $\times 925$.

FIG. 14.—Nucleus with uniformly distributed, distinctly double spireme; $\times 925$.

FIG. 15.—Spireme segmented; 7 pairs of chromosomes shown; $\times 925$.

FIG. 16.—Longitudinal section of microsporangium, showing binucleate, granular tapetal cells and rounded-off microsporocytes in diakinesis; $\times 172$.

FIG. 17.—Nucleus of microsporocyte, showing chromosomes in diakinesis; $\times 925$.

FIG. 18.—Chromosomes in late metaphase of meiosis number one; $\times 925$.

FIG. 19.—Polar view of anaphase of first meiosis; double nature of chromosomes clearly visible; $\times 925$.

FIG. 20.—Four nuclei, each with 15 haploid chromosomes, resulting from second meiotic mitosis; $\times 925$.

FIG. 21.—Transverse section of microsporangium, showing epidermis, endothecium, "middle layers," and tapetum consecutively surrounding tetrads of pollen grains; $\times 172$.

FIG. 22.—Transverse section of anther, showing relative position of 4 microsporangia; $\times 18$.

FIG. 23.—Detail of single microsporangium: epidermis, endothecium, disintegrating tapetum, and tetrads of pollen grains with germinating pores, nucleus, and vacuolated cytoplasm distinguishable; $\times 172$.

FIG. 24.—Transverse section of almost mature anther, showing position of the 4 microsporangia, and partition wall between adjacent sporangia; $\times 18$.

FIG. 25.—Detail of single microsporangium, showing weak spot where split will occur, endothecium with its thickened fibers, and mature pollen grains; $\times 172$.

FIG. 26.—Tetrad of germinating pollen grains; $\times 715$.

FIG. 27.—Median longitudinal section of young pistil; ovules on basal placentula, surrounding growing point of flower; $\times 38$.

FIG. 28.—Single hypodermal archesporial cell of megasporangium; $\times 715$.

FIG. 29.—Archesporial cell in mitosis; $\times 715$.

FIG. 30.—Primary parietal and sporogenous cells; $\times 715$.

FIG. 31.—Similar section but of somewhat differently shaped cells; $\times 715$.

FIG. 32.—Longitudinal but not quite median section of slightly older pistil; $\times 38$.

FIG. 33.—Detail of single ovule, showing procambium, outer and inner integuments, and megasporangium; $\times 172$.

FIG. 34.—Megaspore mother cell in early prophase, with two parietal cells immediately above; $\times 715$.

FIG. 35.—Similar section, showing two megasporangium mother cells; $\times 715$.

FIG. 36.—Nucleus of megasporangium mother cell, just previous to segmentation of spireme; $\times 925$.

FIG. 37.—Chromosomes in diakinesis, 15 pairs visible; $\times 925$.

FIG. 38.—Two megasporangium resulting from first division of mother cell; $\times 715$.

FIG. 39.—Similar stage; nucleus of one cell in mitosis; $\times 715$.

FIG. 40.—Similar stage; nucleus of each cell in different stage of mitosis; $\times 715$.

FIG. 41.—Similar stage; nucleus of each cell in same stage of mitosis; $\times 715$.

FIG. 42.—Similar stage; 4 nuclei in early prophase; no walls formed between grand-daughter nuclei; $\times 715$.

FIG. 43.—Functional megasporangium in micropylar end of ovule; 3 degenerating megasporangium in chalazal end (only 2 shown in this figure, third in next section); $\times 715$.

FIG. 44.—Longitudinal section of 2-nucleate embryo sac; nuclei migrated to each end; cytoplasm containing numerous vacuoles; $\times 715$.

FIG. 45.—Slightly older embryo sac; nuclei in diakinesis; one large vacuole in center; $\times 715$.

FIG. 46.—Longitudinal section of ovule containing 4-nucleate embryo sac; inner integument overtops nucellus, leaving only narrow passage way (micropyle); outer layer of nucellar cells begun to elongate radially about central core of longitudinally attenuated cells; $\times 172$.

FIG. 47.—Detail of single nucleus, showing 15 haploid chromosomes; $\times 925$.

FIG. 48.—Slightly different stage but similar section of 4-nucleate embryo sac; non-functional megasporangium in micropylar end of sac; $\times 715$.

FIG. 49.—Similar section, showing 4 nuclei in mitosis; disintegrating megasporangium in micropylar end; $\times 715$.

FIG. 50.—Eight-nucleate embryo sac; $\times 715$.

FIG. 51.—Mature embryo sac containing synergids, egg, polar nuclei, and antipodal cells; $\times 715$.

FIG. 52.—A, longitudinal, median section of pistil (just previous to fertilization), showing incurved papillae of stigma, stylar canal, and ovules with tips

slightly imbedded in cup-shaped bolsters of placental tissue; *B*, similar section of stigma with two tetrads of pollen grains attached and germinating; $\times 18$.

FIG. 53.—Longitudinal section of embryo sac, showing fertilized egg, and two polar nuclei in process of fusion; $\times 715$.

FIG. 54.—Slightly older embryo sac, showing fertilized egg, synergids containing dark-staining substance, and primary endosperm nucleus; $\times 715$.

FIG. 55.—Fertilized egg and 2 nuclei resulting from first division of primary endosperm nucleus; $\times 715$.

FIG. 56.—Endosperm nuclei in telophase of second mitosis; $\times 715$.

FIG. 57.—Fertilized egg showing 3 of the 4 nuclei resulting from second division of endosperm nucleus; $\times 715$.

FIG. 58.—Longitudinal section of entire ovule, showing radially elongated nucellar cells surrounding central strand of longitudinally attenuated cells, fertilized egg, synergids, and endosperm which is making inroads upon nucellar tissue; $\times 172$.

FIG. 59.—Longitudinal section, showing suspensor cell and hemispherical embryo; $\times 715$.

FIG. 60.—Similar section, mitosis occurring in suspensor cell; $\times 715$.

FIG. 61.—Four-celled embryo; $\times 715$.

FIG. 62.—Somewhat older embryo; dermatogen in process of differentiation; $\times 715$.

FIG. 63.—Longitudinal section of entire ovule, showing 16-celled embryo, outer seed coat with thickened walls, air spaces, and multinucleate endosperm; $\times 172$.

FIG. 64.—Surface section of endosperm shown in preceding figure; nuclei in mitosis surrounded by evenly distributed cytoplasm; $\times 715$.

FIG. 65.—Each nucleus surrounded by denser mass of cytoplasm, strands of which extend to each of neighboring nuclei; $\times 715$.

FIG. 66.—Slightly advanced condition, showing fibrillae between nuclei; $\times 715$.

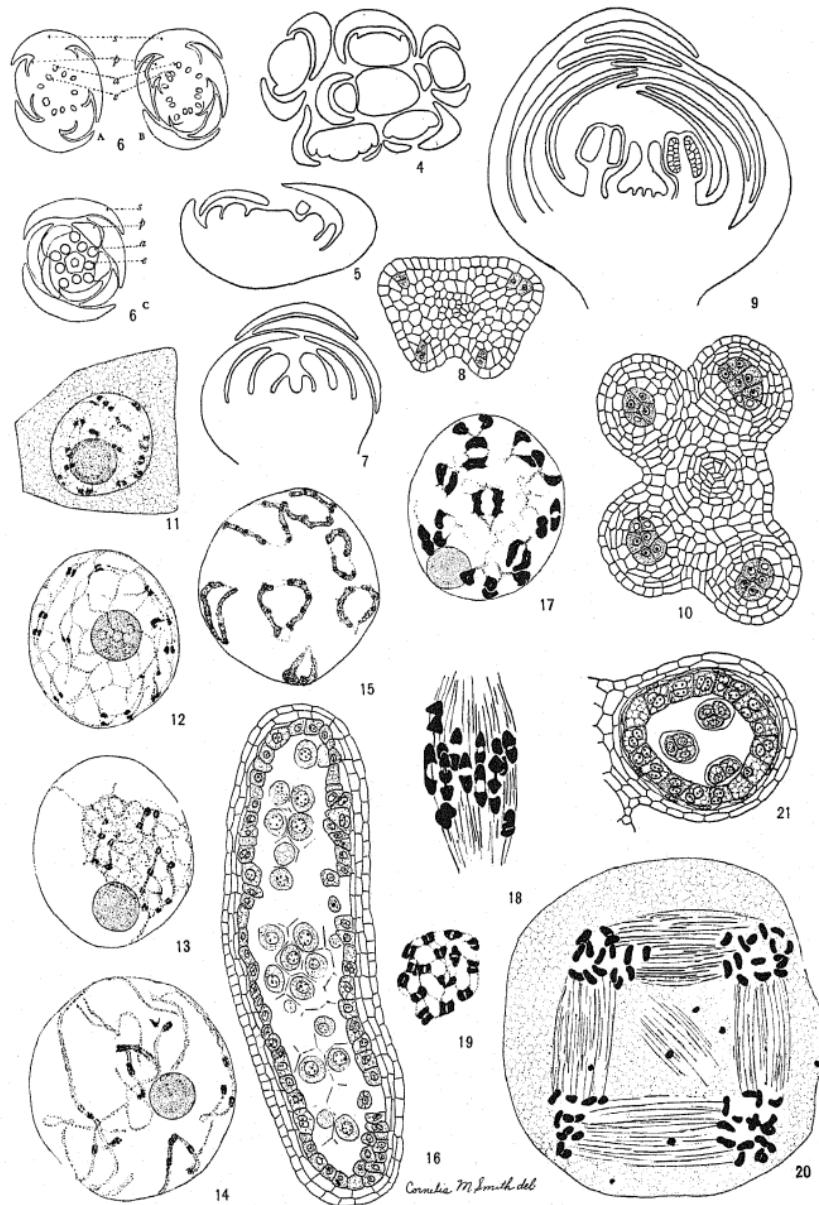
FIG. 67.—Equatorial thickenings on fibrillae; $\times 715$.

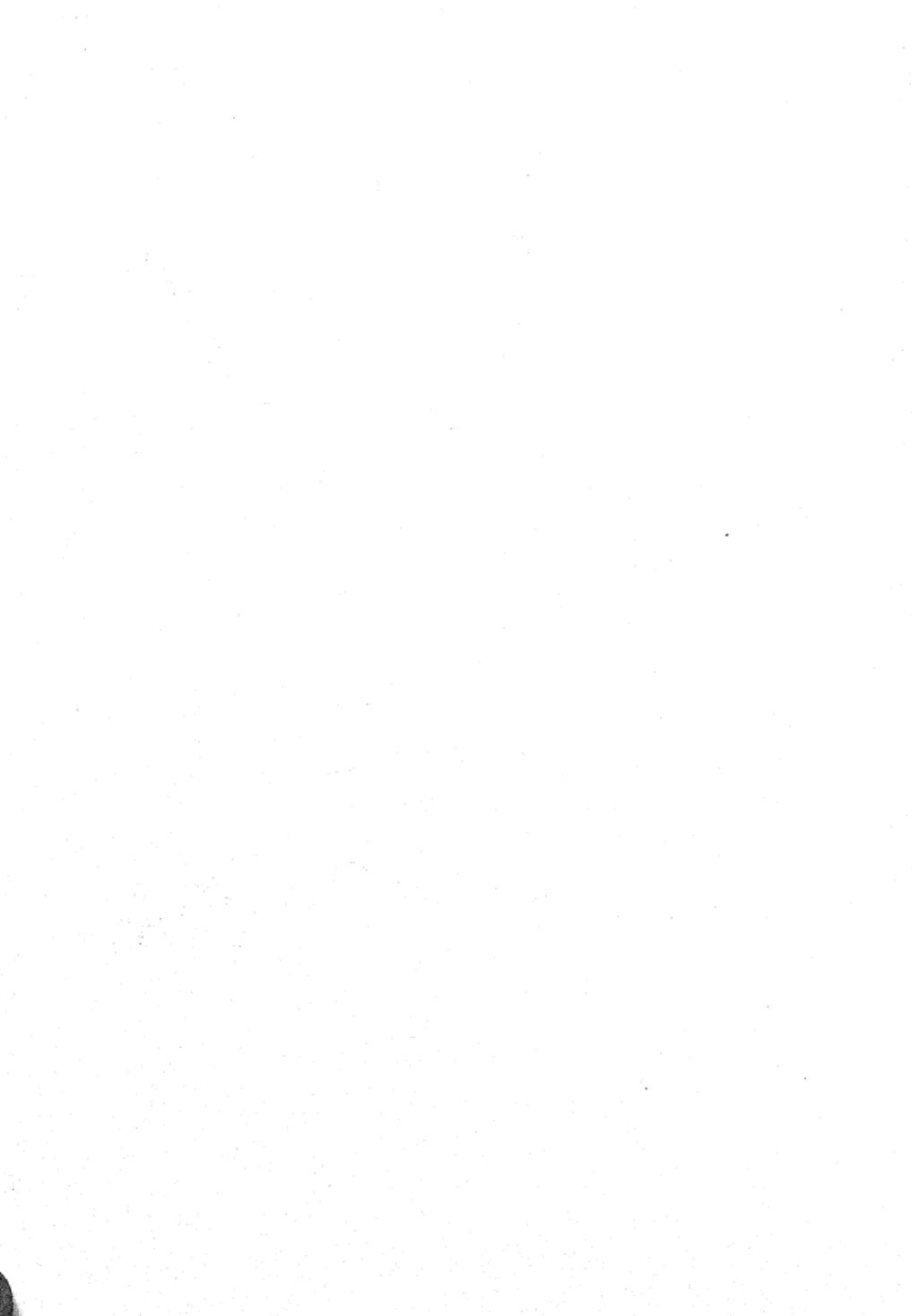
FIG. 68.—Parallel cell plates formed by splitting of equatorial thickenings; $\times 715$.

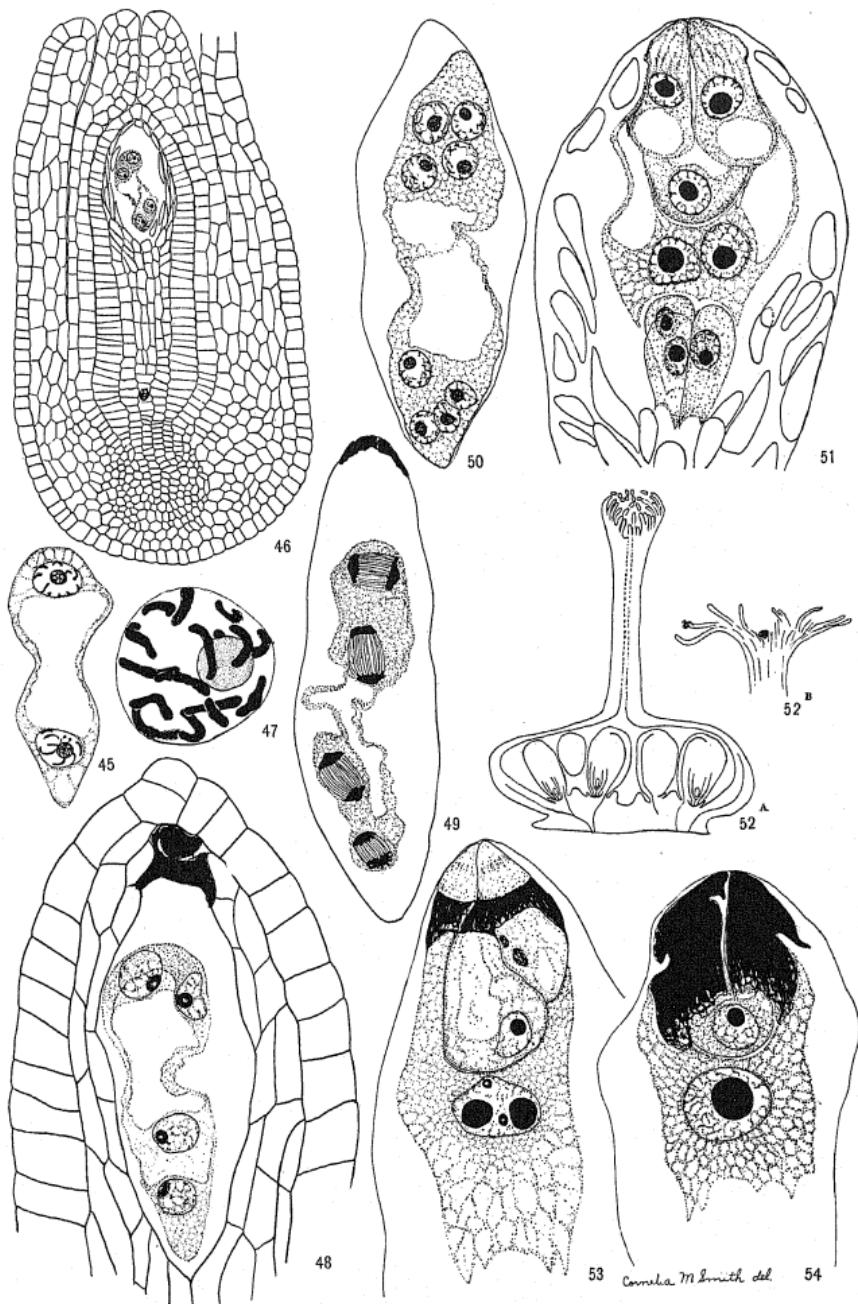
FIG. 69.—Longitudinal section of embryo and endosperm consisting of layer of cells surrounding central sap-filled cavity; $\times 172$.

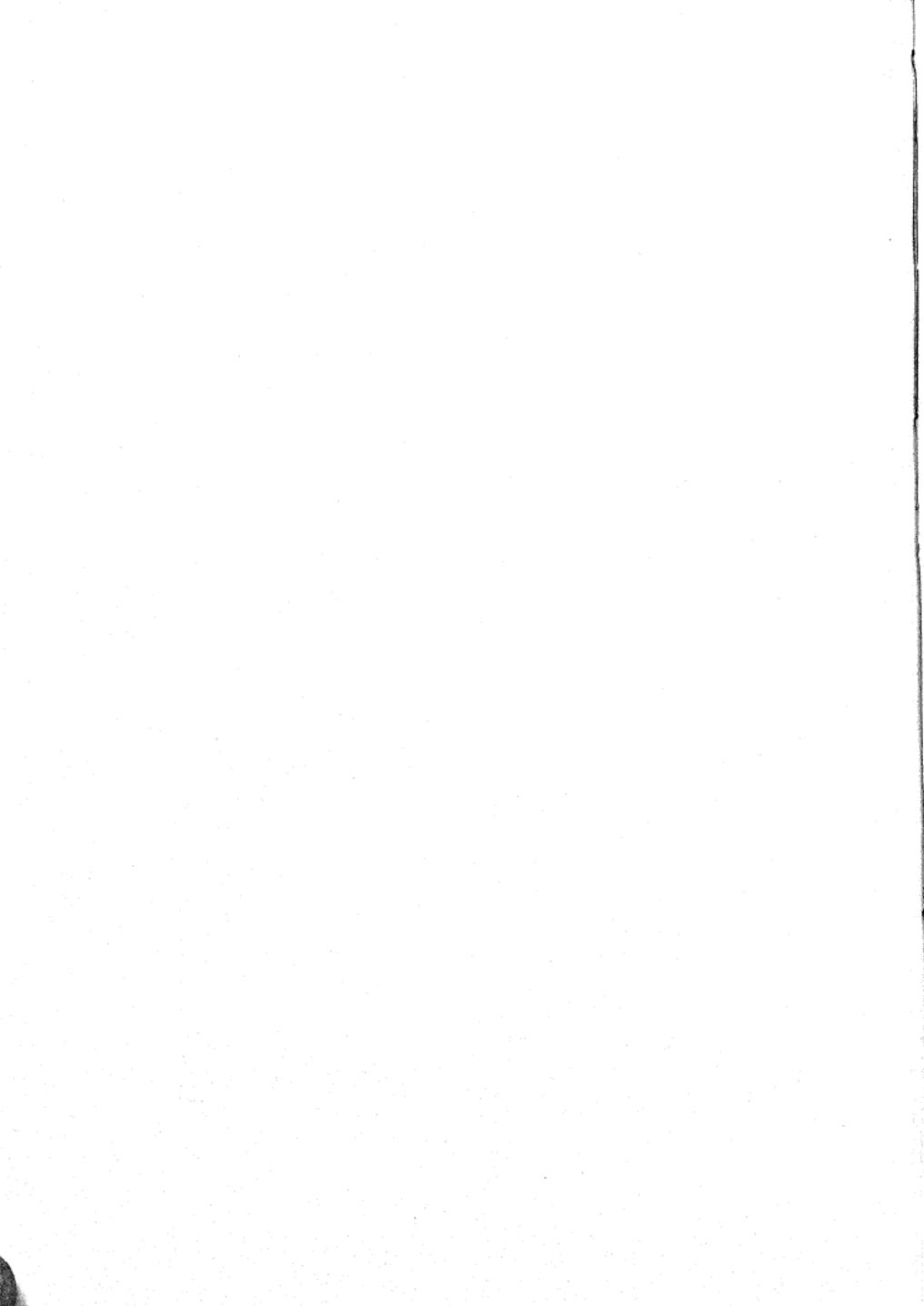
FIG. 70.—Similar section of somewhat later stage, showing seed lid formed by tip of inner integument, and cellular endosperm which completely fills central cavity and surrounds embryo; $\times 172$.

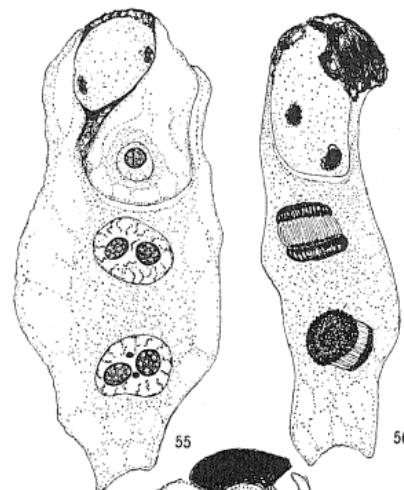
FIG. 71.—Similar section of mature seed, showing embryo consisting of 2 small cotyledons and short radicle surrounded by single layer of cellular endosperm which fills remainder of seed; and thickened bands of cells of outer integument which have coalesced, forming black, shiny seed coat; $\times 73$.



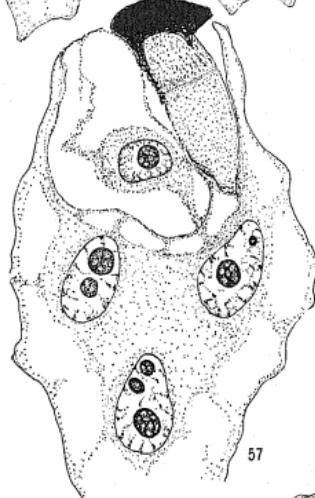




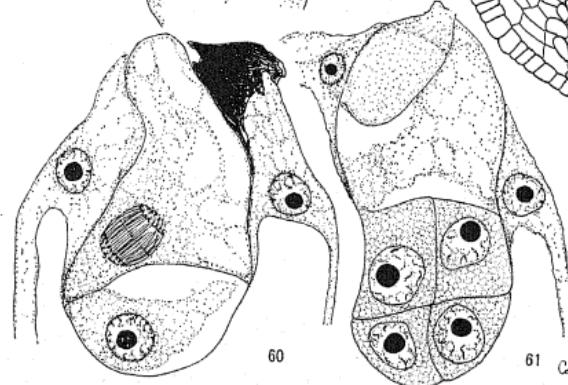




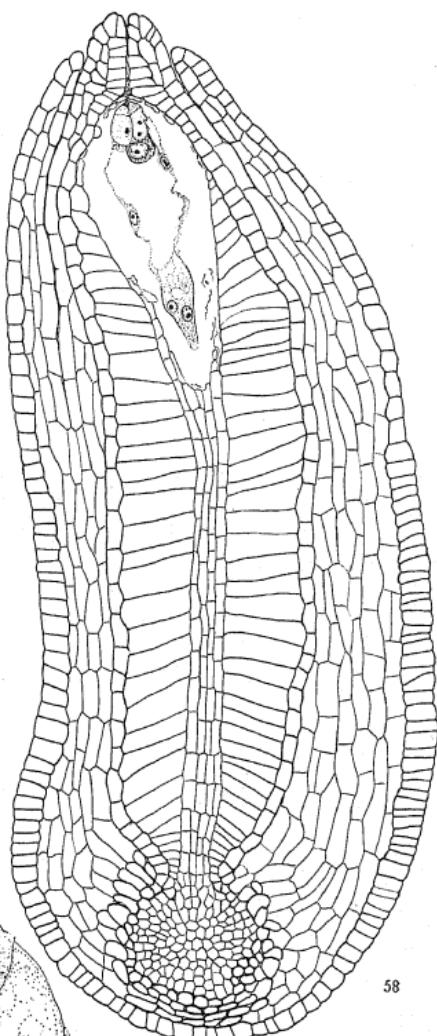
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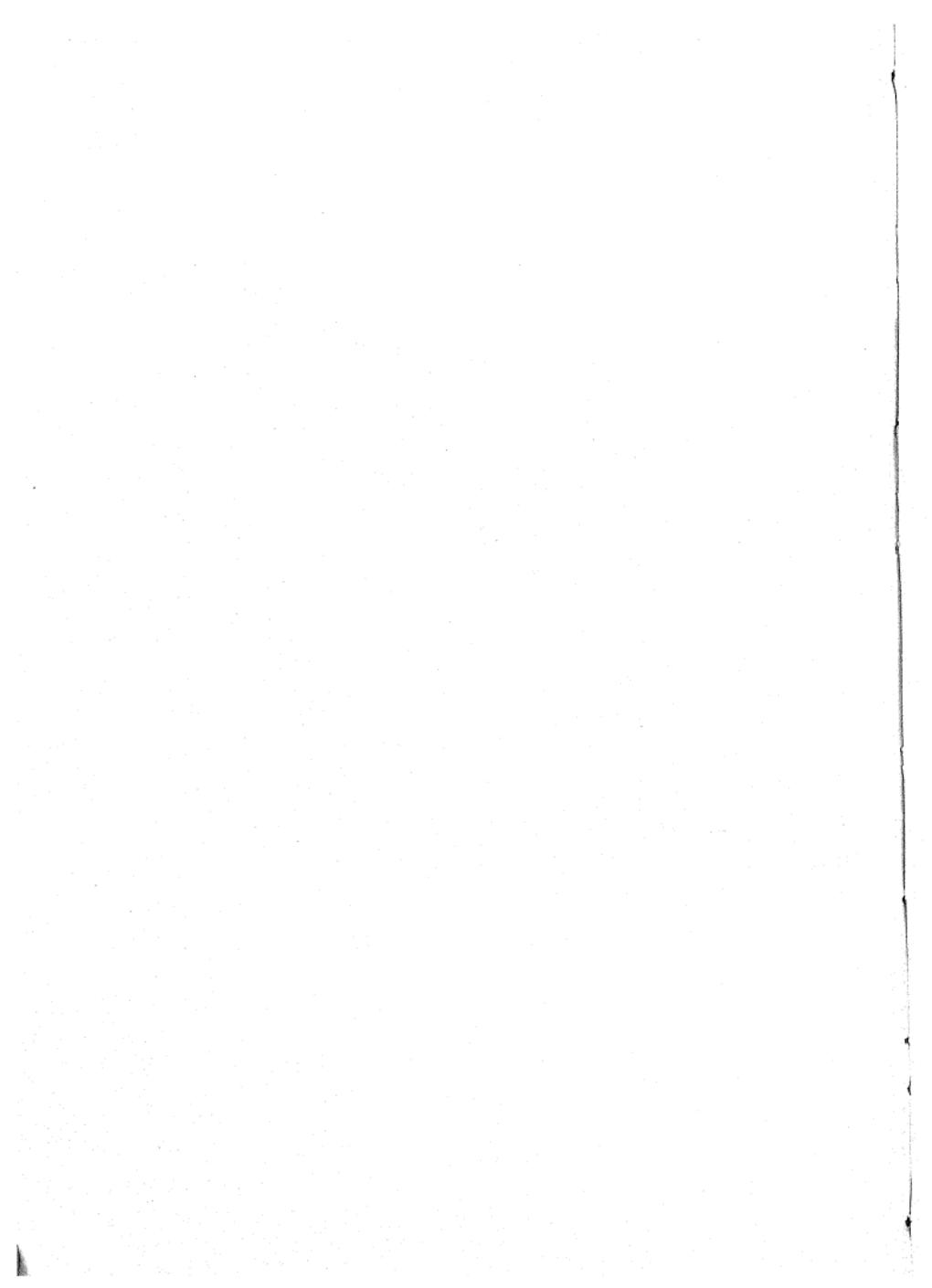
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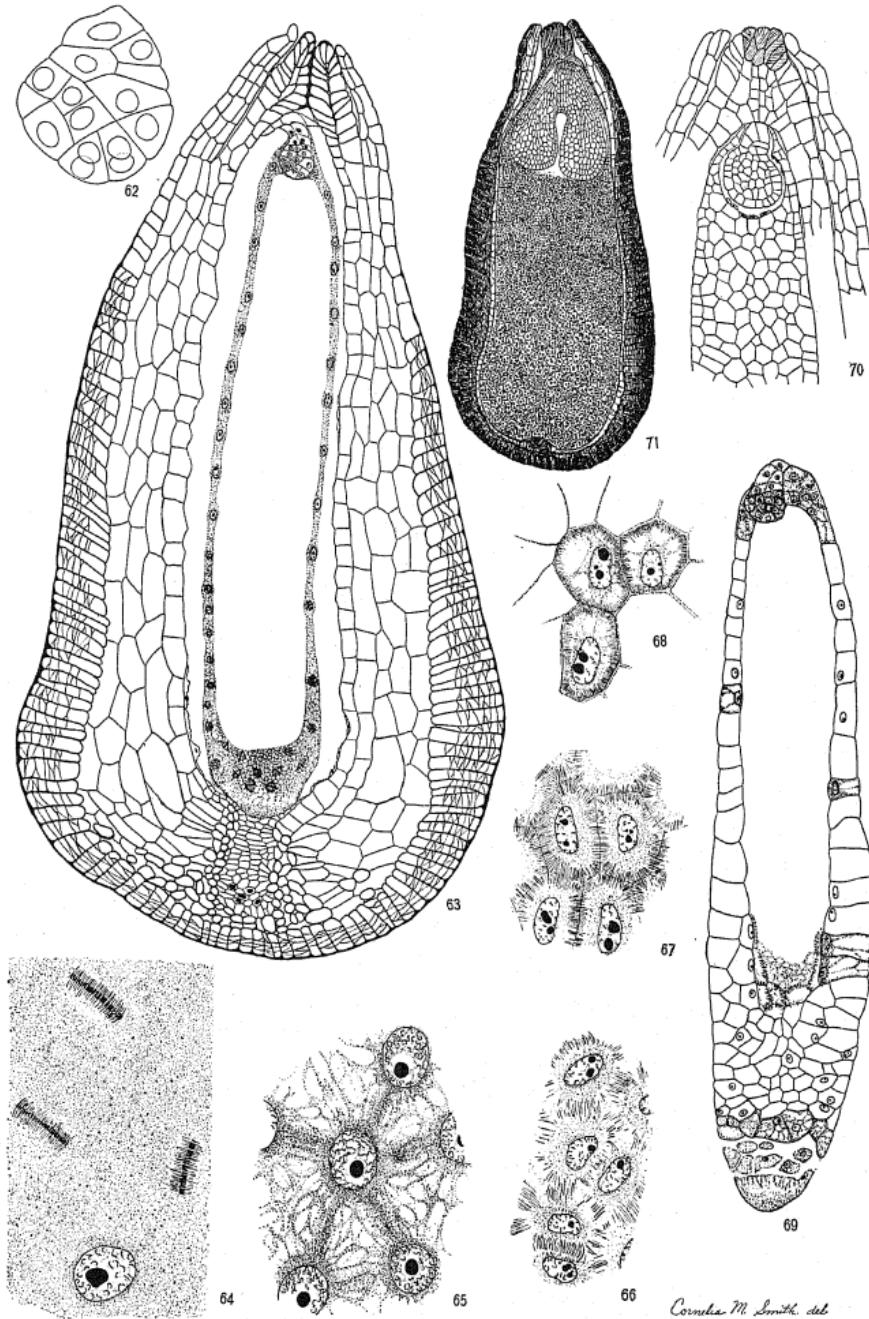


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*Carolina M. Smith del.*

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FURTHER AGGLUTINATION TESTS WITH PHYTOPATHOGENIC BACTERIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 390

G. K. K. LINK, A. E. EDGECOMBE, AND J. GODKIN¹

Introduction

In the course of investigations in this laboratory on the nature of disease resistance in plants, the question arose whether host or symptom specificity of bacterial plant pathogens is correlated with serological specificity. To put the question to experimental test, it was deemed desirable to work with bacteria which morphologically and culturally are practically identical, that is, which according to accepted bacteriological criteria are closely related, but which show decided host or symptom specificity. The yellow pigmented group of which *Bact. campestre* is considered a member was selected. The choice proved fortunate, for it was found that for the organisms studied, with the exception of one (see later), serological specificity as determined by the direct agglutination test is indeed correlated with host specificity (LINK and SHARP 6, SHARP 8, LINK and LINK 5, LINK and TALIAFERRO 7), and even with symptom specificity. The results also indicate that the agglutination test gives promise of usefulness in the identification and classification of bacterial plant pathogens.

It was the purpose of this investigation to continue and extend these exploratory studies. This seemed desirable, in the first place, because in one instance (tests with antisera of *Bact. phaseoli sojense* against suspensions of *Bact. malvacearum*) it was found that cross agglutination was so high that the direct agglutination test was not differentiating. Furthermore, in the previous experiments reciprocal tests were not run with some of the organisms, that is, we did not use antisera of *Bact. cucurbitae*, *Bact. citri*, *Bact. pruni*, and *Bact. X*. Completely reciprocal tests have now been made with all of these

¹ We are indebted to Dr. W. H. TALIAFERRO of the Department of Hygiene and Bacteriology for cooperation in providing space for our experiments and animals.

excepting *Bact. citri*. Another gap in our exploratory studies was due to the fact that none of the cereal pathogens which are supposed to be a part of this group had been included in the tests. One of us (Mr. GODKIN) was making a comparative study of the pathogenicity as well as of the morphological and cultural characteristics of two of these cereal pathogens, *Bact. translucens* and *Bact. translucens* var. *undulosum*, which are reported to differ primarily in their host relations. It was decided not only to determine serologically whether these organisms could be differentiated, but also to determine their relationship to other members of the yellow group. To put the method to a more severe test, we included another yellow organism obtained from Dr. C. S. REDDY, the identity of which was unknown to us, but which was isolated from cereals. This project was undertaken with Mr. GODKIN.

The most intriguing reason for additional studies arose out of our earlier fragmentary study of *Bact. X*. This organism was sent us, after we had reported on our first studies (6), by Dr. E. F. SMITH, with the suggestion that it be included in our experiments. It arrived too late for complete reciprocal tests but was tested against the antisera of various yellow organisms (5). The senior author wrote to Dr. SMITH (March 14, 1927) regarding the relationship of this organism as follows: "Very clearly it is serologically closely related to *Bact. campestris*. It is also closely related to *Bact. phaseoli*, but much less closely." Later, when the paper reporting these results was in preparation, inquiry was made in SMITH's laboratory as to whether the organism was considered a *Bacterium* or a *Bacillus*, and whether it had been named. The following reply was received from Miss LUCIA McCULLOCH: "It was isolated from a leaf spot disease of horse radish and . . . culturally and morphologically it is very close to or identical with *Bact. campestris*."² Later Miss McCULLOCH sent us a culture of another undescribed yellow organism, isolated from poppy by Miss M. K. BRYAN, with the note: "All these organisms, from squash, poppy and horse radish, are very much like *Bacterium campestris* in morphological and cultural char-

² This organism, here designated *Bact. X*, has now (at the time of reading proof) been described as *Bact. campestris* var. *armoraciae* n. var. McCulloch. McCULLOCH, LUCIA, Jour. Agric. Res. 38:269-287. 1929.

acters. We find some slight differences and if you can find other differences in serological tests, it will help us very much in differentiating the organisms." Consequently, we decided to use the direct agglutination test and determine whether it would differentiate between *Bact. X* (horse radish), *Bact. Y* (poppy), *Bact. cucurbitae* (squash), and *Bact. campestre* (cabbage); and also to determine what the test would reveal regarding their relationships to other members of the so-called *Bact. campestre* group. In addition to *Bact. campestre*, *Bact. phaseoli* and *Bact. pruni* were selected as reference points of relationship, because BROOKS, NAIN, and RHODES (3) had reported that *Bact. campestre* and *Bact. phaseoli* are members of one group, but that *Bact. pruni* is not related to either of these or to any of the yellow organisms they studied. This phase of the work was undertaken with Mr. A. E. EDGECOMBE.

To make the results of these two projects more significant, the investigations were combined. Assistance was given by Dr. ADELINE DE SALE LINK, so that we were able to complete the tests of normal sera in one day's run, and the tests with the antisera in another, each of which involved setting up almost 2000 tubes with saline, serum, and bacterial suspensions, and all the necessary preliminary dilutions of sera. By careful organization and division of labor we were able to make about 2000 tests in nine or ten hours of uninterrupted work. The advantage of this method was that all tests were run with the same suspensions, sera or antisera, so that differences due to age of sera, cultures, and density of antigen suspensions were eliminated.

The cultures of *Bact. campestre*, *Bact. phaseoli*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. X* were of the stock used previously (5). *Bact. Y* was obtained from Miss LUCIA McCULLOCH with the note that it had been isolated from poppy by Miss M. K. BRYAN. *Bact. translucens* and *Bact. translucens* var. *undulosum* were of the stock used by Mr. GODKIN in his experiments, and *Bact. Z* was obtained from Dr. C. S. REDDY.

Agglutination tests

The experimental procedure was the same as that reported in earlier papers (5, 7), except for the differences noted here. It was the intention to use antisera produced by six intravenous injections,

but this could not be done consistently because a considerable number of animals were lost at the very beginning of the experiments. Whether these losses were in part due to reaction of the animals to the antigens is not known because some of the uninjected animals also died. We are of the opinion that most of the deaths were due either to an infection which swept through the entire stock or to the extreme heat in the animal room (it was in July and August), or to both. As a result of the loss of some of the injected animals shortly after the first injection, we were confronted with the alternatives of preparing antisera in exactly the same manner and then using antisera of different ages in tests run at one time, of running the tests at different times, or of making the tests at one time but of speeding up the injections of the new animals. The last course was decided upon, and consequently some of the animals were injected intraperitoneally five times with 1 cc. of suspension, the first dose thus being double that of the first intravenous injection, and the others the same as the last five intravenous injections. The animals injected intraperitoneally are starred in the protocol. For all injections, as well as for the suspensions used in the tests, suspensions were prepared to have the turbidity of a standard nephelometer tube, which is supposed to contain 1,800,000 units per cc. As control in all series, a sample of serum drawn before immunization of each animal was tested against suspensions of the organisms to be used in tests against the antisera obtained from that animal. In making the tests, the following final dilutions of antisera were used: 1-80, 1-160, 1-320, 1-640, 1-1280, 1-2560, 1-5120, 1-7680, 1-10,240, 1-15,360, and 1-20,480. The readings were made macroscopically and with a hand lens after incubating the mixtures at 37° C. for one hour in a serological bath, and also after keeping them one hour, two hours, and twelve hours in a refrigerator. The last of these readings are recorded in table I. The protocols of the tests of the normal sera and of the antisera are not given in detail as in previous papers; instead only the titres are indicated, these being taken as the highest dilution of serum or antiserum in which any agglutination occurred as read with the unaided eye and the hand lens. The normal sera which gave agglutination are indicated in table I.

TABLE I
TITRES OF AGGLUTINATION TESTS
ANTISERUM

| SUSPENSION OF ORGANISM (ANTIGEN) | BACT. CAMPSTRE | | BACT. X | | BACT. PHASEOLI | | BACT. CUCUBALAE | | BACT. Y | | BACT. PRUNI | | BACT. TRANS- LUCENS UNDULOSUM | | BACT. TRANS- LUCENS | | BACT. Z | | Sa- LINE CON- TROL | | |
|--|-------------------|---------|---------|--------|-------------------|---------|--------------------|-------|---------|-------|----------------|--------|--|-------|---------------------------|-------|------------|-------|-----------------------------|-------|---|
| | 32 | 28 | 20 | 42 | 34 | 338* | 30 | 39 | 47 | 50 | 4* | 450* | 40 | 41 | 44 | 45 | 48 | | | | |
| Bact. campstre..... | 1-7680 | 1-10240 | 1-1120 | 1-5560 | 1-160 | 1-10240 | 1-5120 | 1-320 | 1-80 | 1-160 | 1-2160 | 1-1280 | 1-640 | 1-320 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. X..... | 1-5120 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. phaseoli..... | 1-640 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. cucubalae..... | 1-320 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. Y..... | 1-320 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. pruni..... | 1-320 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. translucens..... | 1-320 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. undulosum..... | 1-320 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |
| Bact. Z..... | 1-320 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 1-160 | 0 |

* Intraperitoneal injection.

† Very slight agglutination in the normal serum, titre 1-80.

‡ Very slight agglutination in the normal serum, titre 1-160.

§ 0, zero indicates no agglutination.

Absorption of agglutinins

It was found that in several instances higher titres were obtained between antisera and heterologous organisms than between antisera and their homologous organisms. Since in such cases absorption of agglutinins has been found to be of use in separating specific and group agglutinins, we decided to make some preliminary absorption tests to determine whether such tests would give promise of applicability in studies of phytopathogenic bacteria. Because the group dispersed we were not able to carry the absorption test far enough in all cases to obtain unequivocal data. The method arbitrarily adopted was that used by Dr. E. O. JORDAN in his studies of the typhoid and paratyphoid group. We are indebted to Miss IRENE YARROW for information relative to the technique. The antisera to be absorbed were made up to a dilution of 1-50 in saline. These antisera were the same as those used in the direct tests. Then separate lots of 5 cc. of the diluted antisera were pipetted into 2-day old agar slants of the organisms used in the absorption. A sterile piece of cotton was pushed with the pipette over the surface, washing the growth down to the bottom of the slant. The mixture of saline and organisms was then filtered through the cotton several times, by pushing the end of the pipette firmly into the cotton, wedging this against the glass, and alternately sucking the mixture into the pipette and expelling it through the cotton. This gave an even suspension free from clumps of bacteria and agar. After this absorption the mixtures were pipetted into centrifuge tubes, incubated for 2 hours at room temperature, and then centrifugalized. Following this the whole procedure was repeated with the absorbed antisera but with new slants. The clear absorbed antisera were then made up in dilutions of 1-150, 1-300, 1-600, 1-1200, and 1-2400, and reciprocal agglutination tests were run with each of the absorbed sera against the homologous and heterologous organisms. Thus, for example, the antisera of *Bact. campestre* and of *Bact. X* absorbed by *Bact. campestre* and the antisera of *Bact. campestre* and of *Bact. X* absorbed by *Bact. X* were tested against suspensions of *Bact. campestre* and *Bact. X*. The mixtures of absorbed antisera and bacterial suspensions were incubated one hour at 37° C., readings were made, and then the mixtures were placed in a refrigerator and readings made after 1 and

12 hours, as in the direct agglutination tests. The final readings are recorded in table II. The absorption test was applied to *Bact. cam-*

TABLE II
ABSORPTION TESTS

| ANTISERUM OF | TESTED AGAINST SUSPENSION OF | MAXIMUM TITRE | ABSORBED BY SUSPENSION OF | TESTED AGAINST SUSPENSION OF | TITRE (DILUTIONS 1-150 TO 1-2,400) | SALINE CONTROL |
|---|------------------------------|---------------|---------------------------|------------------------------|------------------------------------|----------------|
| A: (Bact. campestre) | (A..... | 1-10240 | A | A | 0* | 0 |
| | B..... | 1-7680 | A | B | 1-300 | 0 |
| | | | B | A | 1-1200 | 0 |
| B: (Bact. X) | (A..... | 1-5120 | A | A | 1-300 | 0 |
| | B..... | 1-10240 | A | B | 0 | 0 |
| | | | B | A | 1-2400 | 0 |
| C: (Bact. translucens) | (C..... | 1-7680 | C | C | 0 | 0 |
| | D..... | 1-7680 | C | D | 0 | 0 |
| | E..... | 1-2560 | C | E | 0 | 0 |
| | | | D | D | 1-150 | 0 |
| | | | D | E | 1-150 | 0 |
| D: (Bact. translucens var. undulosum) | (C..... | 1-2560 | C | C | 1-150 | 0 |
| | D..... | 1-7680 | C | D | 1-150 | 0 |
| | E..... | 1-5120 | C | E | 0 | 0 |
| | | | D | C | 1-150 | 0 |
| | | | D | D | 0 | 0 |
| E: (Bact. Z) | (C..... | 1-320 | C | C | 1-300 | 0 |
| | D..... | 1-5120 | C | D | 1-300 | 0 |
| | E..... | 1-2560 | C | E | 1-1200 | 0 |
| | | | D | C | 1-300 | 0 |
| | | | D | D | 1-300 | 0 |
| | | | E | E | 1-1200 | 0 |
| | | | E | C | 1-300 | 0 |
| | | | E | D | 1-600 | 0 |
| | | | E | E | 0 | 0 |

* 0: Zero indicates no agglutination in dilution 1-150, the lowest used.

pestre and *Bact. X*, for which the direct agglutination test had given differentiation to determine whether it would give sharper differentiation; and to *Bact. translucens*, *Bact. translucens* var. *undulosum*,

and *Bact. Z*, which had not been completely differentiated by the direct agglutination test.

Discussion

In the tests of normal sera slight agglutination occurred in all tubes in which suspensions of *Bact. cucurbitae* were used; however, this never exceeded titres of 1-160. It apparently was due to factors introduced by the contact of sera and antigen, because no agglutination occurred in the saline controls. In two instances very slight agglutination occurred in dilution 1-80 when the normal sera were tested, but not when the antisera were tested (antisera of *Bact. Y* no. 50 and *Bact. Z* no. 48). Very slight agglutination also occurred when the normal sera of animals nos. 30 and 39 were tested against *Bact. translucens* in dilution 1-80; of animal no. 50 against *Bact. X*; of animal no. 41 against *Bact. translucens*; and of animal no. 44 against *Bact. X*. No appreciable difference was noted in the reactions of antisera produced by intravenous and intraperitoneal injection as seen in sera nos. 34 and 33a. Those noted are no greater than the differences obtained with antisera of two animals treated exactly alike, as in the antisera of animals nos. 30 and 39. In the light of our experience, and that of others in the production of antisera with phytopathogenic bacteria, we are of the opinion that experimental work will reveal that intravenous injections give the best titres for some organisms and intraperitoneal injections for others. It seems that dead organisms alone, if they produce any antibodies at all, will not give good titres. It also appears that some organisms when injected while alive are likely to be fatal to the animals. Consequently the safest procedure will be to begin the injections with killed organisms and then change to live organisms in the third injection. Before a final statement can be made a great deal of careful work must be done. This probably will reveal that there is no general rule for phytopathogenic bacteria any more than for other bacteria.

Following the earlier method, we made our final readings of the reactions after 12 hours of refrigeration, during which settling out occurred. In this experiment, in many of the tubes practically no settling out occurred in that time. The flocculi were of the finely granular type rather than of the coarsely granular type previously

encountered in some of the yellow organisms. Whether this difference in the nature of the flocculi in such organisms as *Bact. phaseoli* and *Bact. pruni* indicates that the antisera were less potent than in the earlier experiments, or whether it indicates that changes had occurred in the cultures so that elements which tend to give finely floccular precipitates had come into existence or predominance, we did not have time to determine. *Bact. cucurbitae* flocculated most readily, but this also gave mixtures of the coarse and finely granular type. It is reported, first by WEIL and FELIX (9), ARKWRIGHT (12), and others, that smooth and rough variants of some bacterial colonies give fine and coarse flocculi which settle out slowly and rapidly respectively. The occurrence of a smooth and rough variant in a culture of a phytopathogenic bacterium (*Bact. phaseoli sojense*) has been reported from this laboratory by SHARP (8). There also is evidence that this culture changed. At one time the rough variant of this organism flocculated so easily that it agglutinated spontaneously in distilled water and in saline (8). Later the same organisms gave stable suspensions (4, 5), indicating that its agglutinative properties had changed, possibly because of the origin or the ascendancy of a different element in the culture. If similar changes occurred in the cultures used in these experiments, they were not prominent enough to be detected by change in colony type.³ Considerable variation was noted in the agglutinability of the organisms tested, *Bact. Y* being least, and *Bact. translucens* var. *undulosum* and *Bact. cucurbitae* most readily agglutinated.

Table I shows that ten of the seventeen antisera gave maximum titres with the homologous organisms, namely, *Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. translucens* var. *undulosum*. Accordingly the agglutination test can be used to differentiate and identify these organisms among the other yellow organisms tested. The test failed to differentiate for *Bact. phaseoli*, *Bact. Y*, *Bact. translucens*, and *Bact. Z*.

³ Miss McCULLOCH has reported (at the time of reading proof) rough variants for *Bact. campestre* and *Bact. campestre* var. *armoraciae* (footnote 2). We had noted uneven appearance in colonies of several of the organisms tested (*Bact. campestre*, *Bact. campestre* var. *armoraciae*, *Bact. cucurbitae*, and *Bact. pruni*), and thought at first that we were dealing with contaminations. This proved not to be the case, but we were unable to segregate smooth and rough variants.

In addition, reciprocal reactions were obtained for the following organisms:⁴ *Bact. campestre* and *Bact. X*, *Bact. Y* and *Bact. translucens* var. *undulosum*; *Bact. X* and *Bact. phaseoli* and *Bact. Y*; *Bact. phaseoli* and *Bact. cucurbitae*; *Bact. pruni* and *Bact. translucens* var. *undulosum*; *Bact. cucurbitae* and *Bact. Y*, *Bact. pruni*, *Bact. translucens*, and *Bact. translucens* var. *undulosum* (all of which are more or less questionable because of reactions with normal sera); *Bact. pruni* and *Bact. translucens* var. *undulosum*; *Bact. translucens* and *Bact. translucens* var. *undulosum* and *Bact. Z*; *Bact. translucens* var. *undulosum* and *Bact. Z*. Of the nine organisms tested, *Bact. campestre* showed reciprocal relationship with three others, *Bact. X* with three, *Bact. phaseoli* with four, *Bact. cucurbitae* definitely with one and possibly with four others, *Bact. Y* with two, *Bact. pruni* with two, *Bact. translucens* with two, *Bact. translucens* var. *undulosum* with five, and *Bact. Z* with two.

In an earlier paper (5) no reaction was reported between antisera of *Bact. campestre* and of *Bact. phaseoli* with *Bact. cucurbitae* and *Bact. pruni* respectively. In the experiments reported here reactions occurred between the antisera of *Bact. campestre* and *Bact. cucurbitae* and suspensions of *Bact. pruni*, and between antisera of *Bact. phaseoli* and *Bact. cucurbitae* and suspensions of *Bact. pruni*. We have no explanation to advance for this discrepancy. The results indicate that the agglutinability of the organisms had increased during the time they were in culture. The reaction of *Bact. phaseoli* displayed another striking deviation from the observations of our earlier experiments. Previously in each case its antiserum had given rather high and maximum titres with the homologous organism. In the experiments reported here its antisera gave lower titres than before, and the maximum titres were given with heterologous organisms. One antiserum (no. 34) gave higher titres with three heterologous organisms (*Bact. cucurbitae*, *Bact. translucens*, and *Bact. translucens* var. *undulosum*) than it did with the homologous organism, while the other antiserum (no. 33a, intraperitoneal injection) gave a higher titre with only one heterologous organism (*Bact. cucurbitae*). This deviation may be due to a change in the

⁴ Each reciprocal reaction is listed only once, that is, reciprocal reaction between *Bact. campestre* and *Bact. X*, for example, is listed only under *Bact. campestre*.

culture, possibly because of the origin or the ascendancy of a different element. After these disconcerting results had been obtained we made repeated tests to determine again whether we were in fact working with *Bact. phaseoli*. We found that it was no longer pathogenic to beans, but did find that, according to morphological and physiological tests, it still was *Bact. phaseoli*. Apparently a change in virulence had occurred in the culture. Pathogenicity tests had been made with the culture several months before we made the agglutination tests reported here. It had been found then that the culture was not as virulent as it had been before we made the agglutination tests reported previously (5). If this interpretation of the data is correct, we have an instance of change in virulence of a culture and a parallel change in serological reaction.

It appears therefore that more or less relationship exists between the organisms tested here of the yellow pigmented type represented by *Bact. campestre*. The conclusion that these organisms show group reaction is in part in apparent contradiction with the conclusion of BROOKS, NAIN, and RHODES (3), that no relationship exists between *Bact. pruni* and other yellow organisms tested by them, such as *Bact. campestre* and *Bact. phaseoli*, and also with our earlier tentative conclusion, based on tests which were not reciprocal, in which we concurred with the conclusion of BROOKS and associates (5). It should be borne in mind, however, that these experimenters based their conclusions on data obtained with antisera in dilutions of 1-100, whereas we have incorporated into the summary data obtained with dilutions of 1-80. If we do not consider these titres then we must strike from our list of reciprocal reactions those between *Bact. campestre* and *Bact. translucens* var. *undulosum*; of *Bact. X* and *Bact. Y*; of *Bact. phaseoli* and *Bact. pruni* and *Bact. cucurbitae*; and of *Bact. cucurbitae* and *Bact. pruni*. This would lead to the same conclusion relative to *Bact. pruni* as that of BROOKS, NAIN, and RHODES (3).

While the data indicate that more or less serological relationship exists between those bacteria with yellow pigment which morphologically and culturally seem to be closely related, the serological tests have not revealed whether there is any one parent form. In our experiments *Bact. translucens* var. *undulosum* showed more extensive

relationship than any other organism. This may be due in part to its ready agglutinability. Theoretically, if one had at one's disposal all the extant organisms of this group and could make the serological test quite quantitatively, it might throw some light on the relationship of not only these organisms and their derivation but also possibly on the relationship of their hosts. It is interesting to note in this connection that of the three organisms sent us by Miss McCULLOCH, which are morphologically and culturally scarcely distinguishable from *Bact. campestris*, *Bact. X* (which attacks a member of the Cruciferae) is the most closely related serologically to *Bact. campestris* (which also attacks Cruciferae); and that *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z* (which attack cereals) are more closely related to one another than to any other of the organisms tested.

In the light of the report of Miss McCULLOCH relative to the relationship of *Bact. campestris*, *Bact. X*, *Bact. cucurbitae*, and *Bact. Y*, it is interesting to note that serologically these organisms show varying group reaction. Although *Bact. campestris* and *Bact. X* show very strong group reaction, differentiation nevertheless was possible by dilution of antisera in the agglutination test.

In order to put the question of the relationship of *Bact. campestris* and *Bact. X* to a more severe analysis, the absorption test was applied. Although the results are not absolutely clear-cut, they indicate that while *Bact. campestris* and *Bact. X* are very closely related they are distinct antigenically. That they are closely related is indicated by the fact that high degrees of absorption are obtained when the antisera of both organisms are absorbed by homologous and heterologous organisms. That they are not quite identical is indicated by two facts. On the one hand, absorption of the antiserum of *Bact. campestris* by *Bact. campestris* so completely removed the specific agglutinins that *Bact. campestris* was not agglutinated by this absorbed antiserum in dilution 1-150, and also removed group agglutinins to such an extent that *Bact. X* was not agglutinated by this absorbed antiserum in dilutions above 1-300. On the other hand, absorption of this antiserum by *Bact. X* so completely removed the group agglutinins that *Bact. X* was not agglutinated by it in dilution 1-150, whereas the specific agglutinins were reduced but

gave a titre of 1-1200 when this absorbed antiserum was tested against *Bact. campestre*. The tests with the absorbed antisera of *Bact. X* indicate that the organisms are related, but they do not show whether they are distinct. We were not able to pursue this study further to determine the significance of the titres obtained. The titre of 1-300 obtained when the antiserum of *Bact. X* absorbed by *Bact. campestre* was tested against *Bact. campestre*, probably indicates that absorption was not complete enough to remove the agglutinins.

Bact. cucurbitae apparently is related to both *Bact. campestre* and *Bact. X*, but is less closely related to them than they are to each other. The test also clearly differentiated *Bact. cucurbitae* from *Bact. Y*. *Bact. Y* apparently is related to *Bact. X* but not very closely; it is more closely related to *Bact. campestre*. In fact, the antiserum of *Bact. Y* gave a higher titre with *Bact. campestre* than with the homologous organisms, which may indicate a very close relationship. The higher titre may be due to greater agglutinability on the part of *Bact. campestre*. Unfortunately we did not have time to make absorption tests. Even though the agglutination tests failed to differentiate *Bact. campestre* and *Bact. Y* when the antiserum of *Bact. Y* was used, the serological reactions of the organisms taken as a whole indicate that they differ antigenically. With the exception of *Bact. Y*, these organisms show some relationship to *Bact. phaseoli*. With the possible exception of *Bact. cucurbitae*, their relationship to *Bact. pruni* is very slight. Their relationship to the cereal group also is slight. It appears, then, that though these organisms (*Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, and *Bact. Y*) are antigenically related to form a loose group of varying relationship among one another, and show varying individual relationship to *Bact. phaseoli*, *Bact. pruni*, and the cereal group, they are sufficiently distinct antigenically to permit differentiation by the agglutination test.

When we consider the data relative to *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z*, a very close group reaction is found. The relationship is so strong that the direct agglutination test did not differentiate between *Bact. translucens* and *Bact. translucens* var. *undulosum* when the antiserum of *Bact. translucens* was

used, nor between *Bact. Z* and *Bact. translucens* var. *undulosum* when the antiserum of *Bact. Z* was used. It may be significant in this connection, that *Bact. translucens* var. *undulosum* agglutinates very readily. The agglutination test did differentiate between *Bact. translucens* and *Bact. Z*, and between *Bact. translucens* and *Bact. translucens* var. *undulosum*, when the antisera of *Bact. translucens* var. *undulosum* were used.

The absorption test showed that these organisms are closely related, *Bact. Z* being the least closely related. The test showed clearly that *Bact. Z* is distinct antigenically. It did not differentiate between *Bact. translucens* and *Bact. translucens* var. *undulosum*. Whether this indicates that the absorption test will not differentiate between them or whether more work would have cleared up the situation we do not know. It is apparent from the data that we did not completely absorb when we absorbed the antiserum of *Bact. translucens* with *Bact. translucens* var. *undulosum*, the antiserum of *Bact. translucens* var. *undulosum* with *Bact. translucens*, and the antiserum of *Bact. Z* with *Bact. translucens* and *Bact. translucens* var. *undulosum*. Only *Bact. translucens* and *Bact. translucens* var. *undulosum* show some relationship to the other yellow organisms tested. This is most pronounced with *Bact. phaseoli*, *Bact. cucurbitae*, and *Bact. campestre*, and very slight with *Bact. pruni* and *Bact. Y*. If one considers all the antigenic reactions of the cereal organisms, it is apparent that they are closely related but distinct antigenically.

Summary

1. *Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, *Bact. pruni*, and *Bact. translucens* var. *undulosum* were differentiated by direct agglutination tests with their antisera.
2. Cross agglutination so high that differentiation by the direct agglutination test was not possible occurred between antisera of *Bact. phaseoli* and the heterologous organisms *Bact. X*, *Bact. cucurbitae*, *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z*; between the antisera of *Bact. Y*, and *Bact. campestre*; between the antisera of *Bact. translucens*, and *Bact. translucens* var. *undulosum*; and between the antisera of *Bact. Z*, and *Bact. translucens* var. *undulosum*.

3. Absorption of agglutinins was carried on with the cereal pathogens, and differentiated *Bact. Z* from the others; but, probably because of incomplete absorption, did not differentiate between *Bact. translucens* and *Bact. translucens* var. *undulosum*.

4. Even though the direct agglutination test did not differentiate all the organisms tested, consideration of all the reciprocal reactions of these organisms warrants the tentative conclusion that they are all distinct antigenically.

5. If we consider titres in dilutions of 1-80, group reaction of varying degree was obtained for all the organisms tested, namely, *Bact. campestre*, *Bact. X* (horse radish pathogen), *Bact. phaseoli*, *Bact. cucurbitae*, *Bact. Y* (poppy pathogen), *Bact. pruni*, *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z* (cereal pathogen). *Bact. Z*, *Bact. pruni*, and *Bact. Y* gave the slightest group reactions, in the order named.

6. *Bact. campestre*, *Bact. X*, *Bact. cucurbitae*, and *Bact. Y* antigenically constitute a loose group of varying interrelationship and of varying relationship to the other yellow organisms tested. *Bact. campestre* is very closely related to *Bact. X* and to *Bact. Y*. With the possible exception of *Bact. cucurbitae*, these four organisms are not closely related to *Bact. pruni*, and with the exception of *Bact. Y* they show some relationship to *Bact. phaseoli*. Their relationship to the cereal group is slight.

7. The cereal pathogens *Bact. translucens*, *Bact. translucens* var. *undulosum*, and *Bact. Z* antigenically constitute a closely related group, with varying relationship to the other organisms tested. *Bact. Z* shows the least relationship and *Bact. translucens* var. *undulosum* the most extensive. This relationship is strongest with *Bact. phaseoli*, *Bact. cucurbitae*, and *Bact. campestre*, and slightest with *Bact. pruni* and *Bact. Y*.

8. The exploratory studies of this and the antecedent papers indicate that the agglutination test may find a use in phytobacteriology analogous to its rôle in other fields of bacteriological study. For some organisms the direct agglutination test apparently can be developed to serve in identification of species and strains; for others, absorption of agglutinins will have to be resorted to.

9. These studies also give promise that the agglutination and

absorption of agglutinin tests can be used in grouping and classification of at least some closely related species, varieties, or subvarieties of phytopathogenic bacteria. Before these tests can lead to unequivocal conclusions, even for the group which we have studied, however, considerable more detailed and penetrating work than we have undertaken must be done. In other words, the experimental procedure and the applicability of each method must be determined in reproducible detail for each species and subspecies, and for such groups into which they may fall. Cognizance will have to be taken of such factors as correct titres, varying degrees of agglutinability, spontaneous agglutination, different types of flocculi, etc.

10. Fully as interesting and significant as the few clarifying results here obtained, are the very observations which have introduced factors of apparent uncertainty into some of our experiments. Among these the most significant are the evidences of variation which we have obtained. In addition to the variant of the rough-smooth type (4, 8), the group shows other types of variation whose precise nature was not determined (behavior of *Bact. phaseoli* and others). Study of this phenomenon of variation promises to become an interesting field in itself, in which serological methods may prove as useful as they have in the study of variants in bacteria other than plant pathogens.

UNIVERSITY OF CHICAGO

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MATROCLINY IN FLOWER SIZE IN RECIPROCAL F_1
HYBRIDS BETWEEN *DIGITALIS LUTEA*
AND *DIGITALIS PURPUREA*¹

J. BEN HILL

(WITH THREE FIGURES)

Introduction

Many hybridization experiments have been conducted between *Digitalis purpurea* L. and *Digitalis lutea* L. KÖLREUTER (5), GÄRTNER (2), and FOCKE (1) all worked with this hybrid combination. KÖLREUTER secured F_1 hybrids of *D. purpurea* \times *D. lutea* but failed to secure the reciprocal, although he secured reciprocal F_1 hybrids of the *D. lutea*-*D. thapsi* combination. If *D. thapsi* is actually a distinct species, it is a very close relative of *D. purpurea*. GÄRTNER and FOCKE both secured reciprocal F_1 hybrids of the *D. purpurea*-*D. lutea* combination. GÄRTNER observed two types of plants in the F_1 reciprocal hybrids of the *D. purpurea*-*D. lutea* combination, one type with large yellow flowers and another with flowers differing only slightly from those of *D. lutea*, excepting that they had a reddish tinge. Earlier, KÖLREUTER (5) had observed variations in the F_1 generation of *D. purpurea* \times *D. lutea*, and also unlike reciprocal F_1 hybrids between other *Digitalis* species. FOCKE, who summarized the literature on the topic to 1881, found that reciprocal F_1 hybrids between *D. purpurea* and *D. lutea* differ in flower color, F_1 plants of *D. purpurea* \times *D. lutea* bearing flowers of a more beautiful color than the reciprocal. He states that KÖLREUTER's and GÄRTNER's observations of differences in reciprocals would bear further investigation. Due to the facts that the number of individuals was often small, that very superficial characters (such as slight differences in corolla coloration) were often the basis of comparisons, and that descriptions of so-called natural hybrids of uncertain parentage were fre-

¹ Contribution from the Department of Botany, The Pennsylvania State College, no. 63, published with the approval of the Director of the Agricultural Experiment Station as a partial report on project no. 657. Technical paper no. 454.

quently given undeserved consideration in the descriptions, the earlier accounts are unsatisfactory.

WILSON (9) reported unlike reciprocals with matroclinous tendencies in the F_1 hybrids between *D. purpurea* and *D. lutea*. He reported that the F_1 hybrid plants produce flowers differing as to form and size in the reciprocals, and resembling those of the seed parent to a greater degree than those of the pollen parent. His discussion is primarily of infertile hybrids, and the matter of unlike reciprocals and matrocliny scarcely receives the attention that the subject deserves. WILSON was correct in his observation of unlike reciprocal F_1 hybrids between these species as regards flower size. His discussion of differences of corolla color in the reciprocal hybrids is somewhat misleading, since the observed differences were due to the segregation of corolla color factors in the gametes of the *D. purpurea* parent. *D. purpurea* is generally heterozygous for color factors. When such heterozygous plants are crossed with those of another species bearing yellow flowers, as *D. lutea* or *D. ambigua*, the F_1 hybrids of the heterozygous parents frequently vary among themselves considerably, due to the distribution of the various color factors to the F_1 zygotes.

JONES (4) gives a summary of the literature on species crosses in *Digitalis*, including the *D. purpurea*-*D. lutea* combinations. WARREN (7, 8) has recorded his results on crosses between *D. purpurea* and *D. lutea*. He reported observations on the reciprocal crosses between the parental species, and especially the results of backcrossing the F_1 hybrids to the parental species. He reported fertility of F_1 hybrids when these plants were backcrossed to the parental strains. These results are especially interesting in view of the fact that all previous investigators report total sterility of the F_1 hybrids between *D. purpurea* and *D. lutea*. I have grown several hundred F_1 plants of the *D. purpurea*-*D. lutea* combination, using several strains of both parents, and have never observed the slightest evidence of fertility in any F_1 hybrid of this combination; in fact, I have never seen a fertile F_1 hybrid in which *D. purpurea* was one of the parents, even though species other than *D. lutea* were used in the crosses. WARREN admitted that there was some question as to the taxonomic position of the *D. lutea* which he used in his experiments, and stated that it

was the opinion of Dr. ARTHUR A. HILL of the Kew Gardens that this particular strain is a fertile hybrid between *D. ambigua* and *D. lutea*. The published photographs of WARREN'S *D. lutea* indicate that these plants are very much like a certain small-flowered *D. ambigua* which I received from the Edinburgh Botanic Gardens.

In a recent paper (3) on cotyledon form and size, it was stated that the cotyledons exhibited the only evidence of matrocliny which I had observed in F_1 hybrids between any species of *Digitalis*. The parent species *D. purpurea* and *D. lutea* differ markedly in the shape and size of the cotyledons, and their reciprocal F_1 hybrids show distinct matroclinous tendencies in respect to cotyledon form and size. Since gathering the data for that paper, however, I have grown great numbers of several pairs of reciprocal F_1 hybrids between various strains of *D. purpurea* and *D. lutea*, and now wish to report my observations of matrocliny in flower size in these hybrids.

Investigation

D. lutea bears small yellow flowers whose corollas average about 2.112 cm. in length and 0.619 in width. The numerous strains of *D. lutea*² vary slightly in flower size. The varieties of *D. purpurea* vary in flower color from pure white, white with spots of various colors, to various shades of red, lavender, and purple. The size of the corolla of an inbred strain (45) of *D. purpurea* used in some of the experiments was an average of 4.936 cm. in length and 2.2 cm. in width. Other strains of this species may vary in size from these measurements, but this size is fairly typical of all varieties of the species.

The corolla color of the flowers of the F_1 hybrid between *D. lutea* and *D. purpurea* varies, depending upon the particular colors of the *D. purpurea* parent. If this parent is a white variety without anthocyanin even in the spots in the corolla, the F_1 hybrids are white, the yellow coloration being recessive. If the *D. purpurea* parent is a variety bearing flowers with any degree of coloration, however, the F_1 hybrids will bear flowers with corollas of some shade of purple or

² I wish to acknowledge my indebtedness to the Botanic Gardens of Kew, Dublin, Edinburgh, and Cambridge; the Museum d'histoire Naturelle, Paris; and especially the Jardin Botanico, Madrid, for the collections of seeds of *Digitalis* species used in this study of the hybrids.

red. The degree of color may vary greatly, depending upon the interaction of the genes for corolla coloration.

The reciprocal F_1 hybrid plants of the *D. lutea*-*D. purpurea* combination are indistinguishable in the rosette stage, and bear inflorescences which in general form are identical in appearance (fig. 1). The F_1 hybrids of this combination bear flowers, however,



FIG. 1.—*D. purpurea* (45) \times *D. lutea* (500), and *D. lutea* (500) \times *D. purpurea* (45); inflorescences from reciprocal F_1 hybrid plants.

which differ markedly in size in the reciprocals, and exhibit a distinct matrocliny, resembling the female parent decidedly more than they do the male parent (figs. 2, 3). Measurements³ show that these matroclinous tendencies include both corolla and sepal size (tables I, II). The flower parts differ especially in width.

The most significant data used in this report were taken on hybrid combinations between two varieties of *D. purpurea* and two

³ I wish to give acknowledgments of assistance by Mrs. HELEN D. HILL, Mrs. EDITH HOLMES, and Miss FLORENCE BROWN in accumulating the data herewith presented.

strains of *D. lutea* secured from different sources. An inbred white variety of *D. purpurea* is designated by the number 45, and an inbred

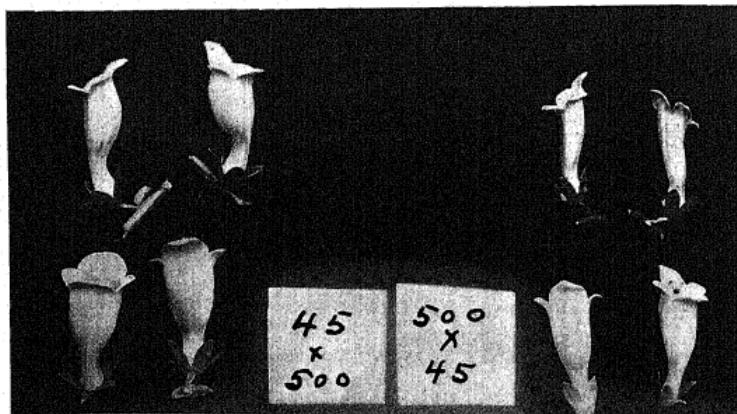


FIG. 2.—*D. purpurea* (45) \times *D. lutea* (500), and *D. lutea* (500) \times *D. purpurea* (45); individual flowers from F₁ hybrid plants.

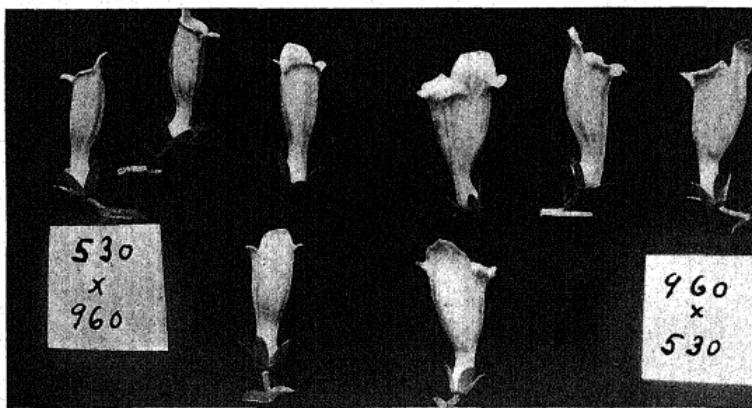


FIG. 3.—*D. lutea* (530) \times *D. purpurea* (960), and *D. purpurea* (960) \times *D. lutea* (530); individual flowers from F₁ hybrid plants.

dark purple variety of the same species bears the number 960. The *D. lutea* strains are designated by the numbers 500 and 530, the latter one having been secured from the Botanic Garden of Edin-

burgh. Other varieties and strains were used in the investigation, and data from the results are incorporated in this report; but reciprocal hybrids involving these strains have yielded the most definite evidence of unlike reciprocals and matrocliny.

TABLE I
COROLLA AND SEPAL LENGTH AND WIDTH IN PARENT SPECIES AND
RECIPROCAL HYBRIDS

| | SPECIES AND RECIPROCAL HYBRIDS | | | | | |
|-------------------------------|---------------------------------|---|---|---|---|---------------------------------|
| | D. LUTEA (535) | D. LUTEA (530) X D. PUR- PUREA (960) | D. PUR- PUREA (960) X D. LUTEA (530) | D. LUTEA (500) X D. PUR- PUREA (960) | D. PUR- PUREA (960) X D. LUTEA (500) | D. PUR- PUREA (45) |
| | Number of measurements (cm.) | | | | | |
| | Corollas 25, sepals 25 | Corollas 25, sepals 25 | Corollas 25, sepals 25 | Corollas 38, sepals 25 | Corollas 45, sepals 25 | Corollas 25, sepals 25 |
| <i>Corolla</i> | | | | | | |
| Average length..... | 1.995 | 2.06 | 3.488 | 2.961 | 3.152 | 4.936 |
| Average width..... | 0.577 | 0.83 | 1.15 | 0.754 | 1.062 | 2.2 |
| <i>Calyx</i> | | | | | | |
| <i>First sepal (smallest)</i> | | | | | | |
| Average length..... | 0.386 | 0.43 | .54 | .53 | 0.59 | 1.15 |
| Average width..... | 0.120 | .14 | .17 | .10 | .13 | .47 |
| <i>Second sepal</i> | | | | | | |
| Average length..... | 0.478 | .56 | .66 | .60 | .60 | 1.44 |
| Average width..... | 0.110 | .19 | .21 | .16 | .20 | .77 |
| <i>Third sepal</i> | | | | | | |
| Average length..... | 0.464 | .53 | .60 | .63 | .68 | 1.14 |
| Average width..... | 0.208 | .27 | .28 | .22 | .27 | .87 |
| <i>Fourth sepal</i> | | | | | | |
| Average length..... | 0.484 | .53 | .59 | .61 | .67 | 1.36 |
| Average width..... | 0.191 | .27 | .28 | .21 | .26 | .88 |
| <i>Fifth sepal</i> | | | | | | |
| Average length..... | 0.462 | .56 | .64 | .60 | .67 | 1.38 |
| Average width..... | 0.118 | 0.19 | 0.21 | 0.16 | 0.19 | 0.76 |

The difference and the matroclinous tendencies in flower size in the reciprocal F_1 hybrid plants are well illustrated in the case of *D. lutea* (530) \times *D. purpurea* (960). The difference is clearly shown in fig. 3, and in the measurements of flower sizes in reciprocals (tables I, II). The hybrid *D. lutea* (530) \times *D. purpurea* (960), whose seed parent is the smaller-flowered *D. lutea*, bears flowers which are dis-

tinctly smaller than those of the hybrid *D. purpurea* (960) \times *D. lutea* (530), whose seed parent is the larger-flowered *D. purpurea*. The average measurements of corolla length are 2.96 cm. for the former and 3.488 cm. for the latter, and of corolla width 0.83 cm. and 1.15 cm. respectively (table I). *D. purpurea* (960) \times *D. lutea* (530) bears

TABLE II
PERCENTAGE DIFFERENCES OF COROLLA AND SEPAL SIZE IN
RECIPROCAL HYBRIDS

| RECIPROCAL HYBRIDS COMPARED | NO. OF MEAS- UREMENTS | PERCENTAGE DIFFERENCE | | | | | | | | | | | |
|---|--------------------------------|-----------------------|-------|------------------------------|-------|-----------------|-------|----------------|-------|-----------------|--------|----------------|-------|
| | | COROLLA | | CALVX | | | | | | | | | |
| | | | | FIRST SEPAL (SMALLEST) | | SECOND SEPAL | | THIRD SEPAL | | FOURTH SEPAL | | FIFTH SEPAL | |
| | | Length | Width | Length | Width | Length | Width | Length | Width | Length | Width | Length | Width |
| <i>D. purpurea</i> (960) \times <i>D. lutea</i> (500) | 25* | 6.50 | 40.84 | 11.32 | 30.0 | 15.0 | 25.0 | 7.94 | 22.73 | 9.83 | 23.81 | 11.66 | 18.75 |
| <i>D. lutea</i> (500) compared with <i>D. lutea</i> (500) | 25† | | | | | | | | | | | | |
| <i>D. purpurea</i> (960) \times <i>D. lutea</i> (530) | 25 | 17.83 | 38.50 | 25.58 | 21.42 | 17.85 | 10.52 | 13.20 | 3.70 | 11.32 | 3.70 | 14.29 | 10.52 |
| <i>D. lutea</i> (530) compared with <i>D. lutea</i> (530) | 25 | | | | | | | | | | | | |
| <i>D. purpurea</i> | 25 | | | | | | | | | | | | |
| Total of all <i>D. purpurea</i> \times <i>D. lutea</i> | 50‡ | | | | | | | | | | | | |
| Total of all <i>D. lutea</i> \times <i>D. purpurea</i> | 164 | 8.96 | 43.59 | 10.66 | 37.49 | 8.15 | 2.90 | 5.07 | 24.73 | 6.23 | -13.17 | 9.50 | -0.18 |

* Corollas 45.

† Corollas 38.

‡ Corollas 70.

flowers which are 17.83 per cent longer and 38.5 per cent broader than the reciprocal (table II).

D. purpurea (960), in combination with another strain of *D. lutea* (indicated by the number 500), produced F_1 hybrids bearing flowers with the same matroclinous tendencies and to about the same degree; that is, 6.5 per cent difference in length and 40.84 per cent difference in width of corolla (table II).

Similar differences, also with matroclinous tendencies, may be seen in the measurements of sepal length and width (tables I, II). The sepals were measured, designating the smallest sepal as the first and the others in rotation as second, third, fourth, and fifth respectively. As in the corolla size, there is a greater difference in width than in length of sepal in the flowers of these particular hybrid combinations.

The totals of all measurements secured for flower size in the *D. lutea*-*D. purpurea* F₁ reciprocal hybrids, when grouped for comparison (table II), show a difference for corolla length of 8.96 per cent and for corolla width of 43.59 per cent. These differences in both instances indicate matrocliny. Certain of the sepal measurements for the total group fail to indicate matrocliny and are designated by a minus sign.

Summary

Reciprocal F₁ hybrids between *Digitalis lutea* and *D. purpurea* are unlike and matroclinous in respect to flower size. These differences appear in both corolla and calyx.

THE PENNSYLVANIA STATE COLLEGE
STATE COLLEGE, PA.

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BRIEFER ARTICLES

THREE NEW DINOFAGELLATES FROM NEW JERSEY¹ (WITH TWELVE FIGURES)

The dinoflagellates from brackish waters of New Jersey, herewith described, have been observed in connection with studies on the food of oysters which have been in progress for some years. *Prorocentrum triangulatum* is certainly, and *Amphidinium fusiforme* is probably of great importance in this connection. *Polykrikos barnegatensis* is evidently rare, but represents an interesting addition to a curious and not too well known genus.

Prorocentrum triangulatum sp. nov.

Cell strongly compressed, triangular as seen from side, rounded behind, left valve with a delicate tooth. Surface of valves covered with minute poroids, the margins appearing striate. Chromatophores yellow-brown, irregular, commonly broken up into rather small masses. Length (without tooth) 17-22 μ .

Extremely common in Barnegat Bay, where it is sometimes the most abundant organism in oysters' stomachs; also in Delaware Bay. This is obviously one of the thirteen organisms listed by GRAVE² as forming the bulk of the food of Chesapeake Bay oysters. He illustrates it but gives it no name. It is difficult to believe that so common and abundant a form as this has been undescribed, but I cannot place it in any recorded species. Dr. MARIE LABOUR, who has been kind enough to examine the description and drawings, also expresses the opinion that it is undescribed. I venture, therefore, to describe it as a new species.

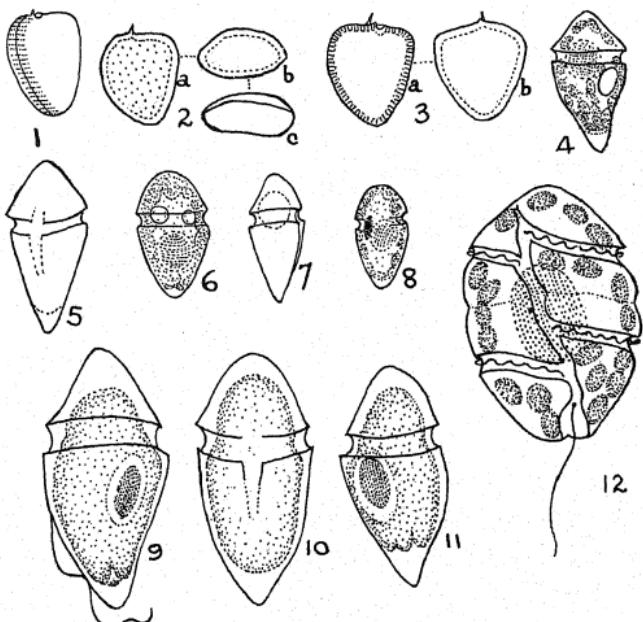
Amphidinium fusiforme sp. nov.

Cell fusiform, about twice as long as broad, circular in cross-section. Epicone hemispherical-conical; girdle anterior; hypocone two to two and one-half times as long as the epicone, long conical or somewhat helmet-shaped; sulcus obscure, but apparently encroaching upon the epicone

¹ Publication no. 15, New Jersey Oyster Investigation Laboratory. Paper of the Journal Series, New Jersey Agricultural Experiment Station.

² GRAVE, C., Fourth report of the shell-fish commission of Maryland. pp. 324-348. Baltimore. 1912.

and extending at least half way from the girdle to the antapex. Body packed with yellowish green chromatophores except at the posterior end, which is hyaline; a dull orange stigma is visible in ventral region beneath



FIGS. 1-12.—Figs. 1-3, *Procentrum triangulatum*: fig. 1, right dorsal; fig. 2 (a) right lateral, (b) optical section from anterior end, (c) posterior view of same individual; fig. 3, right and left sides of same individual; $\times 110$. Figs. 4-11, *Amphidinium fusiforme*: fig. 4, right lateral, from life, $\times 1060$; fig. 5, ventral, from life, $\times 1060$; fig. 6, dorsal, from life, $\times 1060$; fig. 7, right lateral showing sulcus, killed in strong IKI and preserved in formalin, $\times 110$; fig. 8, ventral, from life, showing stigma, $\times 1060$; figs. 9-11, left lateral, ventral and right dorsal respectively, killed in osmic acid, followed by chrom-acetic and preserved in formalin (plasmolysis of contents permits clear view of delicate theca), $\times 2436$; fig. 12, *Polykrikos barnegatensis*: ventral, from living specimen (anterior longitudinal flagellum not seen), $\times 1060$. All figures drawn with camera lucida at magnifications indicated and reduced one-fifth in reproduction.

the girdle. Nucleus central and dorsal, mostly behind the girdle. The entire body incased in a delicate pellicle, usually visible only in fixed specimens in which the contents of the cells have become plasmolyzed.

Length usually 17-22 μ ; diameter 8-11 μ ; sometimes larger or smaller. The smallest individual measured was 12.6 \times 7.3, the largest 31 \times 13.6 μ .

In shape, size, structure, and the possession of a pellicle, close to *A. crassum* Lohmann, but smaller, proportionately narrower and more fusoid, and possessing chromatophores.

Barnegat Bay, common; Delaware Bay, exceedingly abundant locally, occurring in dense swarms in late summer and giving rise to the phenomenon of "red water."

Polykrikos barnegatensis sp. nov.

Body ovate, nearly circular in cross-section, slightly concave on ventral side, composed of two zooids, closely united, with a very slight constriction between the individual zooids and a single large beaded nucleus in center; girdle or each zooid a descending left spiral, displaced twice its width; sulcus extending from just below the anterior end to the posterior end, which is slightly 2-lobed. Ground protoplasm colorless, but containing numerous oval, yellow-brown chromatophores. Nematocysts lacking; evidently autophytic. Length 46 μ ; diameter 31.5 μ .

Barnegat Bay, September 7, 1928. But one individual seen, which was observed in living condition for nearly half an hour, when it underwent cytolysis.—G. W. MARTIN, *State University of Iowa, Iowa City*.

[Accepted for publication November 2, 1928]

CURRENT LITERATURE

BOOK REVIEWS

Tree books

The increasing interest of the public in the larger woody plants of the country is shown by the increasing number of books on trees and shrubs. It seems to be the ambition of every state to have a tree book of its own, and Minnesota¹ and Kansas² have just issued their volumes.

The former is the more pretentious, taking the form of an illustrated volume and including shrubs as well as trees. Its interpretation of terms is so liberal as to include cultivated as well as native trees, and to extend the idea of shrubs to such half woody forms as species of *Spiraea* and *Ceanothus*. Its illustrations consist of good drawings of the leaves and flowers of many of the species described. Perhaps its most notable features are the number and elaborateness of its keys. These should make the identification of species a comparatively easy matter.

The Kansas book appears more modestly, in paper covers, but contains almost as many pages, more illustrations, and quite as much subject matter. A third of the volume is devoted to economic uses of trees, and is illustrated by numerous photographs, which include tree habits, tree trunks, and tree industries. In the second part is a manual of trees, illustrated by drawings already familiar to us in *Michigan trees*. It includes many species, like white pine and Douglas fir, that are not native to Kansas, indeed are scarcely planted within its borders. It is well suited to its purpose of increasing interest in tree study and tree planting.—G. D. FULLER.

The problem of fermentation

A little monograph on alcoholic fermentation, written two years ago by SCHOEN³ of the Institute Pasteur, has just been issued in English translation. A chapter on the rôle of hydrogen in biological oxidation has been added especially for this edition, and is the clearest statement of the nature of cell oxidations which the reviewer has seen. The whole monograph is characterized

¹ ROSENDAHL, C. O., and BUTTERS, F. K., *Trees and shrubs of Minnesota*. 8vo. pp. 385. figs. 146. Univ. of Minn. Press, Minneapolis. 1928. \$4.

² SCOTT, C. A., and GATES, F. C., *Trees in Kansas*. 12mo. pp. 372. figs. 209. Report, Kansas State Board of Agric., J. C. MOHLER Secretary. Topeka, Kansas. 1928.

³ SCHOEN, M., *The problem of fermentation. The facts and the hypotheses*. Transl. by HIND, L. H. pp. xii+210. London: Chapman and Hall. 1928.

by unusual lucidity, and is an admirable summary of the present status of our knowledge of fermentation by one who has worked at the problem for many years. There is a good bibliography.—H. S. WOLFE.

NOTES FOR STUDENTS

Peat bogs and postglacial vegetation.—Most of our forest trees produce an abundance of pollen. This wind-borne pollen seems to be rather evenly distributed over forested areas, and falling into lakes and bogs it is there preserved on account of its resistant exine, and may be recognized in lake and peat deposits thousands of years later. The relative amounts of such fossilized pollens from different tree species are believed to give a rather accurate picture of the forest vegetation of the area at the time deposits were being laid down. Because of this hypothesis there has developed an active study of peat deposits by means of pollen analyses. Its beginning in Sweden early in the present century and its spread into various other European countries has already been noted in this journal.⁴ More recently similar studies have been undertaken in America.

Working with assistance afforded by the International Education Board and the Geological Survey of Canada, AUER⁵ has made a survey of some thirty-four bogs in southeastern Canada, ranging in their distribution from Nova Scotia to Niagara, Ontario. He differentiates bogs of oceanic and continental types dependent on existing climatic conditions. The bogs of the oceanic type are found principally in Nova Scotia and New Brunswick, under the influence of high atmospheric humidity. They seldom result from the filling up of lakes; they have frequently a convex surface; they are dominated by *Sphagnum*; and have usually very uniform peat deposits. In many instances their lower strata show marine deposits. The bogs of the continental type, on the contrary, very commonly come from the filling up of lakes or ponds; they have flat or concave surfaces; they are largely dominated by *Carex*, with *Amblystegium* formerly very important; and their peat deposits lack the uniformity of oceanic bogs. Various transitional forms are distinguished, connecting the two main types.

AUER's classification of peat deposits, while not entirely new, is logical and useful. The four classes are: (1) Limnistic peat, that might also be called lake peat, consisting of rather amorphous ooze that may be either inorganic or organic. The former may contain traces of plankton while the latter is made up largely of remains of Diatomaceae and Desmidiaceae. In addition, there is at times a detritus ooze containing numerous remains of such submerged aquatics as *Ceratophyllum*, *Myriophyllum*, and *Naias*. This peat frequently occurs as lens-shaped deposits near the former shores of filled lakes. (2) Telmatic or marsh peat, which is a form in which *Carex* is the chief constituent and which is found

⁴ BOT. GAZ. 83:323-325. 1927.

⁵ AUER, VÄINÖ, Stratigraphical and morphological investigations of peat bogs of southeastern Canada. Communicationes. Inst. Quaest. Froestalium Finlandiae Editae 12:1-62. 1927.

as a thinner or thicker layer in almost all bogs. It is particularly abundant in bog deposits of the continental type. This *carex* peat may be either quite pure or may contain mixtures of the remains of such plants as *Eriophorum vaginatum*, *Amblystegium*, or *Sphagnum*. (3) Semi-terrestrial peat; this is essentially sphagnum peat often forming thick strata that are exceptionally pure and homogeneous. (4) Terrestrial peat; this is deposited from bog forests and consists of sphagnum peat together with tree stumps and the remains of trees and shrubs.

With respect to the nature of the stratified deposits, AUER distinguishes three types of bogs. They are sphagnum peat bogs, composed of semi-terrestrial or terrestrial peat; *carex* peat bogs, with deposits of telmatic peat; and a combination of these in which different peats are variously stratified.

AUER's investigations were conducted with the usual technique of peat boring and pollen analysis. The various bogs visited are described in detail as to their appearance and stratification. Diagrammatic cross-sections of profiles are given for eight of the more important, with pollen diagrams for twelve. Variations are found that are widespread, and show distinctly warmer and cooler periods which are synchronous throughout the area studied. The changes are similar to those that have obtained in Europe, and the conclusion is that the BLYTT-SERNANDER classification of postglacial periods may also be applied in Canada. The evidence from this investigation seems to show that an early, dry, warm, boreal period was followed by a well marked atlantic period with moist warm climate. This was followed in turn by a drier sub-boreal period and a cooler subatlantic period, both rather poorly marked. The further study of AUER's material may serve to clear up many doubtful points and emphasize the need of further investigations.

ERDTMAN, who has been one of the most active workers in this field, has recently brought together the extensive literature on the subject,⁶ together with a plate illustrating the principal tree pollens and a map showing where pollen analyses have been carried out in Europe. An extensive atlas and key to all pollens likely to be found in bogs has been compiled by MEINKE,⁷ and should be of material assistance to workers in this field.

ERDTMAN has also been extending his investigations in the British Isles,⁸ in Holland,⁹ and in Belgium and France,¹⁰ confirming results that were formerly

⁶ ERDTMAN, G., Literature on pollen-statistics published before 1927. *Geolog. Fören. Stockholm Förhandl.* **49**:196-211. 1927.

⁷ MEINKE, HERBERT, *Atlas und Bestimmungsschlüssel zur Pollenanalytik. Bot. Archiv.* **19**:380-449. 1927.

⁸ ERDTMAN, G., Studies in the postarctic history of the forests of northwestern Europe. I. Investigations in the British Isles. *Geolog. Fören. Stockholm Förhandl.* **50**:123-192. 1928.

⁹ ——, Studien über die postarctische Geschichte der nordwesteuropäischen Wälder. II. Untersuchungen in Nordwestdeutschland und Holland. *Ibid.* **50**:368-379. 1928.

¹⁰ ——, Études sur l'histoire postarctique des forêts de l'Europe Nord-ouest. III. Recherches dans la Belgique et au Nord de la France. *Ibid.* **50**:419-428. 1928.

announced, while RUDOLPH¹² has summarized the extensive investigations in Bohemia, and FURRER¹³ has added to those in Switzerland. These all go to prove the validity of the BLYTT-SERNANDER series of climatic periods, and are rapidly reconstructing the details of the vegetation of each period.

Studies in Russia,¹⁴ however, seem to show that the variations in climate were there less clearly defined, although at least one period warmer than the present is evident, with a more northern distribution of such species as *Quercus* and *Pinus*.

Still more recently similar methods, applied by GISTL¹⁵ to some interglacial deposits in Germany, show this last interglacial epoch to have been similar to the postglacial, in having periods corresponding to the preboreal, boreal, atlantic, and sub-boreal with similar vegetation. He estimates the duration of the time represented by these interglacial deposits at 11,000-12,000 years.

These results were similar to those obtained by SZAFER¹⁶ from studies in various localities in central Europe. It would seem that the ice age preceding the interglacial period dealt with was one of a severity much beyond that of the last glacial period.

Finally, WOODHEAD¹⁷ has carefully reviewed the evidence upon which these studies have been based, and concludes that pollen analyses, especially when considered in conjunction with the results from other branches of postglacial research, provide a valuable accumulation of data from which the past history of the forests of Europe may be reconstructed with considerable accuracy. WOODHEAD's most valuable contribution is a table in which the various researches have been summarized for British Isles, Denmark, Sweden, Norway, Finland, and Switzerland.—G. D. FULLER.

¹² RUDOLPH, KARL, Die bisherigen Ergebnisse der botanischen Mooruntersuchungen in Böhmen. Beihefte Bot. Centralb. 45:1-80. 1928.

¹³ FURRER, ERNST, Pollenanalytische Studien in der Schweiz. Beiblatt Vierteljahrsschr. Naturf. Gesell. Zurich 72: no. 14. 1-38. 1927.

¹⁴ GERASIMOV, D. A., Climatic changes and forest development during the post-glacial period, according to peat bog studies. Bull. Jard. Bot. Princ. U.R.S.S. 25:319-362. 1926.

¹⁵ GISTL, RUDOLF, Die letzte Interglazialzeit der Lüneburger Heide. Pollenanalytisch betrachtet. Bot. Archiv. 21:648-711. 1928.

¹⁶ SZAFER, W., Über den Charakter der Flora und des Klimas der letzten Interglazialzeit bei Grodno in Polen. Bull. Internat. Acad. Polonaise. Series B. 1926.

¹⁷ WOODHEAD, T. W., The forests of Europe and their development in postglacial times. Empire Forestry Jour. 7:1-18. 1928.

THE
BOTANICAL GAZETTE

June 1929

CYTOTOLOGICAL AND OTHER FEATURES OF VARIANT
PLANTS PRODUCED FROM X-RAYED SEX CELLS
OF *Nicotiana tabacum*

T. H. GOODSPED

(WITH ELEVEN FIGURES)

As already reported (3), large numbers of variant individuals occur in selfed progeny from X-rayed sex cells of *Nicotiana tabacum* var. *purpurea*. In the present report a more detailed description of the external morphology of certain variant types and particularly of their cytological condition is included. An analysis of second and third generation progenies of variants is being made and will be the subject of a subsequent report. Plants from sex cells exposed to radium emanations and from seeds and seedlings subjected to ultra-violet, radium, and X-rays are being grown.

NATURE AND IMMEDIATE EFFECTS OF TREATMENT

During January, and again during October, 1927, pot-grown plants of *N. tabacum* var. *purpurea* (U.C.B.G. 014), comparable in size with the one shown in fig. 1a, were taken from the greenhouse and placed under a Coolidge tube housed in a fan-cooled lead room. Previous to exposure all open flowers and seed capsules were removed, leaving buds of all sizes, from those extremely minute to those less than 12 hours from anthesis. Distance from the target in the case of individual buds was subject to some variation, despite

efforts by trimming and tying to orient all buds in one plane. The average distance was 30 cm. At the January X-raying one plant was irradiated for 10 minutes and another for 20 minutes, while during the October X-raying two plants were exposed each for 15 minutes. On a number of occasions during the past year plants of other *Nicotiana* species have been irradiated under identical conditions, except that time and target distance have been varied.

In every case a standard, unshielded, deep therapy tube was employed at an effective potential of 50 kv. The current through the tube was 2.8 milliamperes.

In the case of both plants of the January X-raying, most of the smaller flower buds were abscised within 48 hours. Immediately after treatment aceto-carmine smears of anthers from buds of various sizes were taken. Practically all the buds containing P.M.C. stages were abscised, whereas all minute buds up to 4 mm. over-all were retained along with all large buds containing maturing pollen and E.M.C. in all stages. All these buds matured normal flowers and in every case set seed on self pollination. Without going into the evidence in detail, it should be noted that incidence of bud abscission at such stages is not a criterion of effective dosage. The data in hand indicate that the relation of irradiation to the abscission reaction and factors controlling or modifying it should provide an interesting field for carefully controlled investigation.

The four plants involved in the two X-rayings showed no visible somatic modification, and there was no abscission of later and adventitious buds on the terminal inflorescence or on the inflorescences subsequently produced on laterals. One plant of the October X-raying exhibited almost at once an acceleration of growth, and at times treated plants of other *Nicotiana* species have given striking evidence of the same phenomenon. Statements in the literature as to the relation of growth to irradiation are often contradictory, and again the interest of careful investigation of this problem suggests itself. *N. rustica* might prove to be useful material in such case, since on three different occasions pot-grown plants of variety *pumila* irradiated just previous to first flowering have shown an immediate and very marked growth acceleration.

The statements just made as to the absence of any visible somatic

modification following irradiation hold for all treated species except *N. langsdorffii*. In this case all buds of the terminal inflorescence were abscised immediately after treatment and all the first flowers on laterals were abnormal. After a short time only normal flowers were produced, however, and both abnormal and normal flowers set full capsules of seed. A possibly comparable "recovery" has been referred to in the literature dealing with both the physiological and genetic effects of irradiation.

TABLE I
OCCURRENCE OF VARIANTS IN TEN POPULATIONS FROM SELFED SEED PRODUCED BY
X-RAYED SEX CELLS OF NICOTIANA TABACUM VAR. PURPUREA (U.C.B.G.014)

| NO. | CULTURE NO. | EXPOSURE (MINUTES) | CONDITION OF BUD | NO. VARIANT | NO. NORMAL | TOTAL | PERCENT- AGE VARIANT (APPROX- IMATE) |
|-------------|-------------|-----------------------|-----------------------------|----------------|---------------|-------|--|
| 1..... | 27151 | 10 | ♀ prophase (?) | 26 | 169 | 195 | |
| 2..... | 155 | 10 | ♀ prophase (?) | 10 | 188 | 198 | |
| 3..... | 149 | 10 | 24 hours before anthesis | 4 | 28 | 32 | 7 |
| 4..... | 153 | 10 | Anthesis | 7 | 183 | 190 | |
| 5..... | 150 | 20 | ♀ I-M (?) | 33 | 152 | 185 | |
| 6..... | 154 | 20 | ♀ I-M (?) | 136 | 32 | 168 | |
| 7..... | 152 | 20 | 24 hours before anthesis | 12 | 61 | 73 | 50 |
| 8..... | G27101* | 15 | ♀ I-M (?) | 10 | 63 | 73 | |
| 9..... | 110* | 15 | ♀ I-M (?) | 3 | 33 | 36 | |
| 10..... | 112* | 15 | ♀ prophase (?) | 4 | 35 | 39 | 11 |
| Totals..... | | | | 245 | 944 | 1189 | 20 |

* These three progenies were from seed following the October X-raying and flowered in the greenhouse from December to February. The conditions under which they grew made it difficult to estimate the expression of plant characters, particularly vegetative ones. They are included in tables I and II merely to indicate that the second X-raying of *purpurea* gave rise to variants of the same type as those which occurred in the previous experiment.

OCCURRENCE AND NATURE OF VARIANTS

During the summer of 1927, 1040 plants from selfed seed of the January X-raying were grown, in seven populations. In the greenhouse during the past winter three populations from selfed seed of the October X-raying were grown (table I, nos. 8, 9, 10). In both cases plants differing in practically all characters from the control occurred and are referred to as "variants." Table I shows the occurrence of variant plants in the various populations. For assistance

in classification of the field populations I am indebted to Miss PRISCILLA AVERY.

In the greenhouse a population (G₂₇₁₀₂) from control 014 ♀-X-rayed ♂ was grown. The pollen matured from a bud in which

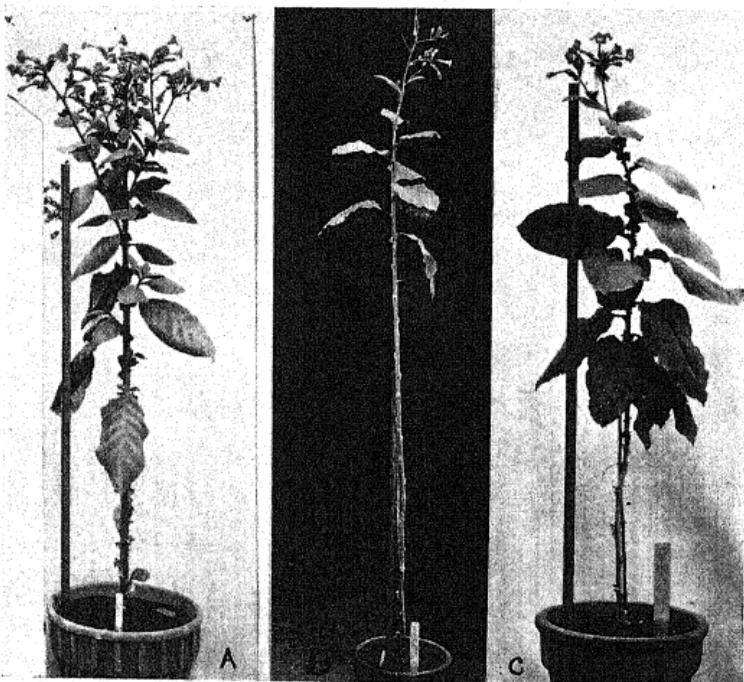


FIG. 1.—*a*, 27014, *Nicotiana tabacum* var. *purpurea*, approximately equivalent in condition to plants which were irradiated; *b*, *c*, G_{27101P71} and 110P₅, variants from a repeat X-irradiation of 014 (table II, nos. 17 and 18).

the pollen was, apparently, almost mature when X-rayed. Six quite striking variants appeared in a population of forty-four plants. This result suggests that irradiation of mature pollen may effect genetic modification, a point which should prove of some importance.

Often variant characteristics were clearly shown in early seedling stages, and usually the mature plants from variant seedlings were also strikingly variant in external morphology. For example, in 27154 (table I, 6), of eighteen abnormal seedlings held in pots in the



FIG. 2.—Plants from X-rayed sex cells: *a*, 27154P004, extreme variant type containing fragmented chromosomes (figs. 8, 9, 10); *b*, 27150P003, equivalent to control and showing 24_{II} at I-M.

greenhouse, sixteen gave variants at maturity. Two, on the other hand, gave apparently normal plants, and in a number of instances it has been observed that variant seedlings of abnormally slow growth produced vigorous basal laterals from which a mature plant, normal in external appearance, was built. In general, conspicuously slow early growth in the case of young plants of normal appearance

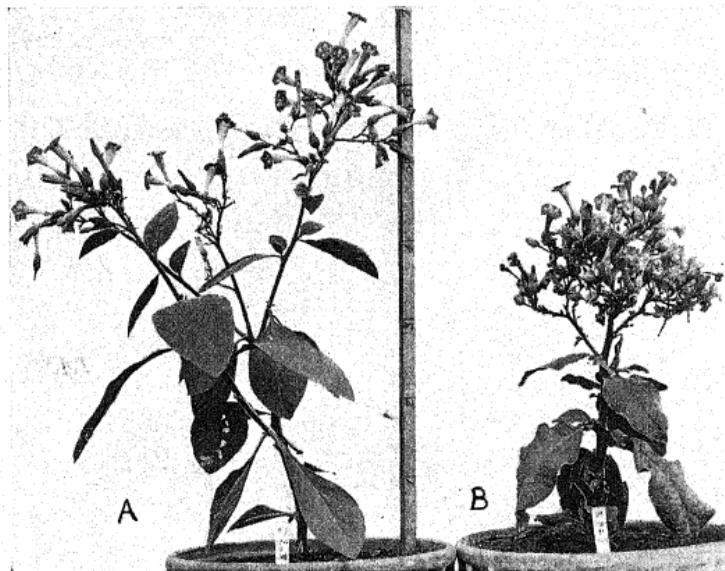


FIG. 3.—Plants from X-rayed sex cells: *a*, 27154P014; *b*, 27150P001 (table II, nos. 6 and 2).

seems to be a fairly reliable index of variant character at maturity. In rare instances very rapid maturity has occurred and has usually been correlated with variation.

An attempt is made by figs. 1-6 and by the data included in table II to give some picture of the extent of variation in all plant characters exhibited by the many variants produced from selfed seed of flowers which matured from X-rayed buds.

It is obviously difficult to express many of the data included in table II in any but relative terms. It should be clear, however, that in the plants listed the distinction from the control in character ex-

pression was sharp, and in the majority of cases involved most of the vegetative and floral characters. In addition to the characters listed, many variations in leaf number, leaf shape, venation, position of the floral organs, lobing of the stigma, number of carpels, and so forth, were noted. The fertility data in table II are of relative significance and are based upon field observations of capsule development

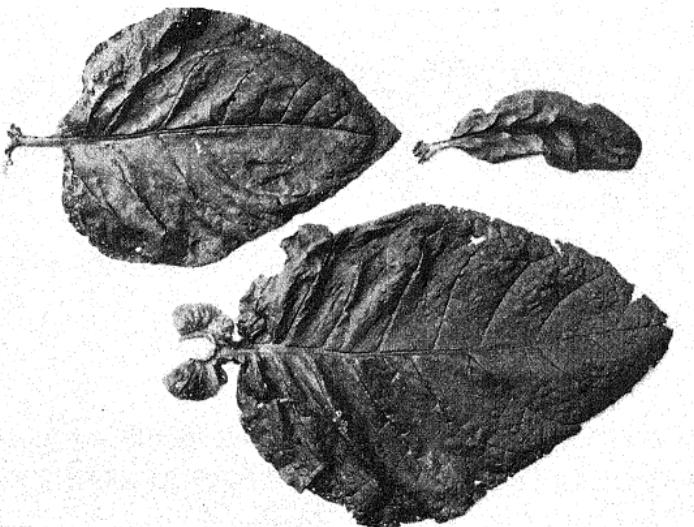


FIG. 4.—Variation in leaf shape: 014 (control) at bottom; 27154P80 (table II, no. 10) above, left; 27154P177 (table II, no. 15) above, right.

and contents. The results of crosses to the control and of germination studies of variants and normals indicate that, as might be anticipated, various degrees of gametic and zygotic lethality are involved.

The results of irradiation in these experiments have involved effects, presumably largely nuclear, which express themselves upon the immediate progeny. In the case of plants apparently no such effects, comparable in extent and character of resulting variation, have been hitherto reported. The figures given in table I indicate that it may be possible to place the relation of X-ray dosage for sex

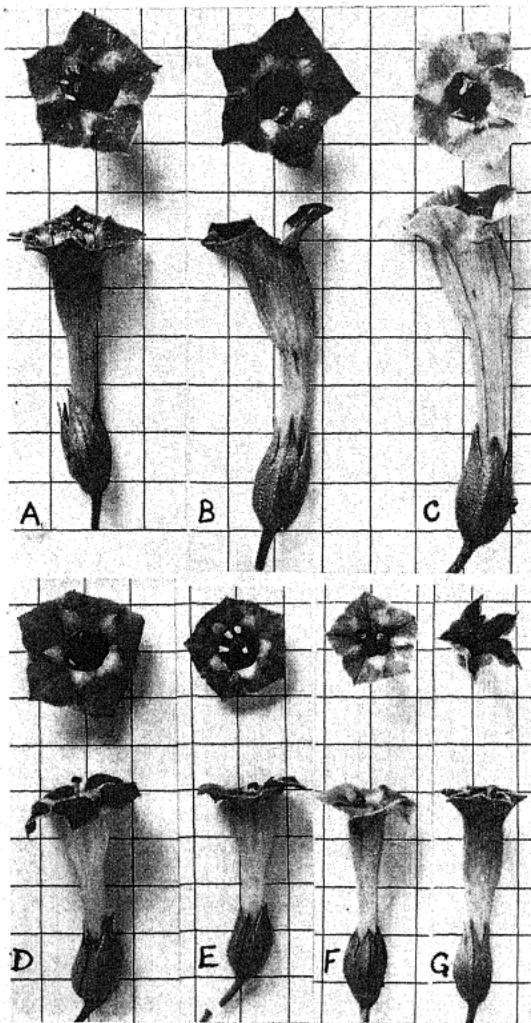


FIG. 5.—Variation in flower form: *a*, 014 (control); *b*, 27154P168, flower larger than 014 and color darker; *c*, 27154P23, larger and color lighter; *d*, G27110P5, flower smaller and stigma exserted; *e*, G27101P46, smaller and color darker; *f*, 27154P158, smaller and color lighter; *g*, 27154P129, smaller, "limb erect" type.

cells to incidence of variation in immediate offspring, on at least a semiquantitative basis. The increase in total number of variants with increase in length of exposure would appear to have some defi-

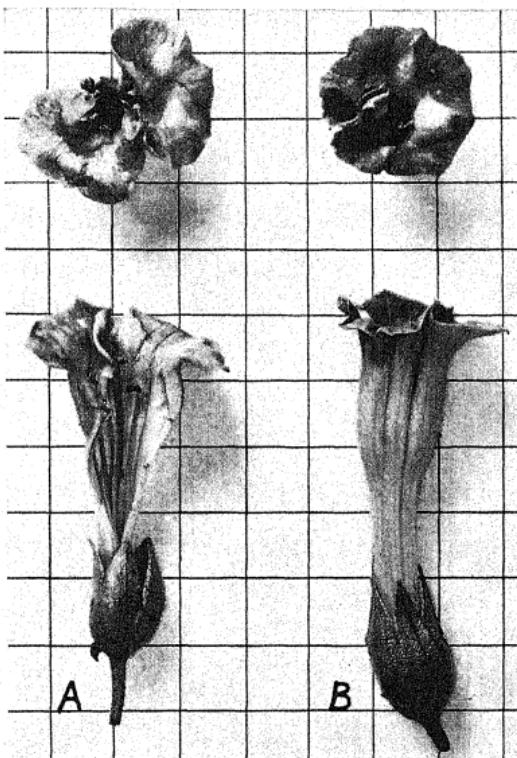


FIG. 6.—Extreme variation in flower form: *a*, 27151P200, "split" type; *b*, 27154P142, "pleated" type.

nite significance. A similar direct relation appears to exist where the condition of the sex cells during treatment was the same. Contrasting the occurrence of variants from buds in which the pollen and embryo sacs were mature (table I, 3, 4, 7), the 10-minute exposure gave approximately 5 per cent whereas the 20-minute exposure gave approximately 15 per cent of variants. A similar contrast in the case

TABLE II

| No. | CULTURE NO. | FIGURE NO. | FLOWER SIZE (MM.) LENGTH: SPREAD | FLOWER COLOR | FLOWER FORM | HABIT | LEAF | FERTILITY (PERCENT-AGE) | CYTOLOGY AT I.M. | REMARKS |
|-----|---------------------------------|----------------------------|----------------------------------|---|----------------------------------|--|-------------------------------|-------------------------|----------------------------|--|
| 1. | 2701* (Central) 2715xPoc1 | 1a, 45, 53 75, 1b 3b | 59:36 42:34 | Red Red | Normal Slender and fluted | Dwarf Medium Incised limb Extra lobes | Sessile Sessile Sessile | 100 0-25 | 24n | Leaves thick, gray green |
| 2. | Poc3 | 1b, 11c 3a, 8a, 9, 10c | 59:32 39:24 45:33 | Red, dark red Red, dark Light red | Normal Normal Incised limb | Dwarf Medium Dwarf | Sessile Sessile Sessile | 100 0 0-50 | 24n 23n+1f 23n+2f(?) | Equivalent to control Leaves gray green, linear others thick, stigma and anthers low |
| 3. | Poc4 | | | | | | | | | Leaves lanceolate |
| 4. | Poc4 | 3a | 59:28 67:38 | Light red Pink | Tube slender Normal | Medium Medium | Petiolate Petiolate | 0-75 | 24n | Very large flower |
| 5. | Poc4 P23 | 5c 5c | 36:38 | Red | Tube slender | Dwarf Dwarf | Sessile Sessile | 0 | 23n+1f 23n+2f | |
| 6. | P24 | 8b | 39:25 | Red | Tube fluted | Medium | Petiolate | 0-25 | 24n+1f 23n+1f(?) | Thick, linear leaves |
| 7. | P36 | | 39:26 | Red | Normal | Medium | Petiolate | 75 | 24n | |
| 8. | P80 | 4 | 53:29 | Red | Limb erect | Medium | Sessile | 25 | | |
| 9. | P129 | 5g | 49:25 | Red | Normal | Short | Sessile | 75 | | Small leaves and flowers |
| 10. | P129 | 5f | 49:25 | Light red | Limb erect | Short | Petiolate | 75 | 23n+1f 23n+2f(?) | Large leaves |
| 11. | P138 | 5f | 37:22 | Light red | Normal | Dwarf | Sessile | 25-30 | 23n+1f 23n+2f(?) | Stigma low |
| 12. | P138 | 7b, c 5b | 55:31 57:41 | Lively red Dark, lively red | Limb erect | Medium | Petiolate | 25-30 | 23n+1f 23n+2f(?) | |
| 13. | P168 | 4 | 47:35 | Light red | Broad | Medium | Petiolate | 0 | Variable | Venation irregular and weak |
| 14. | P168 | 5e | 35:24 | Dark, lively red | Limb fluted | Medium | Sessile | 75 | | Internodes short, auricles large |
| 15. | Gatrot 46 | 1b | 58:35 | Light red | Calyx reduced | Tall | | | | Very tall, fast growing |
| 16. | Gatrot 46 | 1c, 5d | 45:30 | Light red | Limb erect | Dwarf | Petiolate | 25-30 | | Stigma exerted |

* For *N. tabacum* var. *purpurea* (or 4) the details of habit, leaf shape, and flower form may be obtained from figs. 1a, 4, and 5a.

of immature pollen and E.M.C. in division stages (table I, 1, 2, 5, 6) shows approximately 10 per cent for the shorter and 45 per cent for the longer exposure. As might be anticipated, the percentage of variants from buds where one set of sex cells was in meiotic stages was higher than from buds in which both sets were practically mature.

These data as to the relation of exposure time and stage of sex cell development to the production of variation are obviously too fragmentary to permit the drawing of larger conclusions. On the other hand, it is doubtful to what extent it will be possible in the future to supplement them in a significant fashion in the case of the material used. One major difficulty would seem to be the variations in the stages of maturity of sex cells in a given bud. A small ovary of *tabacum* contains from 1000 to 1500 E.M.C. in all stages, from early prophase to second telophase. In the case of P.M.C. the series is not so extensive, but it is apparently readily extended by changes in temperature, and perhaps by other factors also. From this point of view alone, since the character, extent, and significance of genetic modification following irradiation would appear to be intimately related to the stage of maturity of the given sex cell, the problem of relation of dosage to incidence of a particular category of variation is complicated. In this connection, the possible use of mature pollen should be considered, particularly in the light of the results just noted.

CYTOTOLOGICAL FEATURES EXHIBITED BY VARIANTS

Some forty plants of 27150, 154, and 155 (tables I, II) were studied cytologically. In the majority of cases meiotic stages were examined in detail, while in a few only pollen tetrad counts were made. In most cases both aceto-carmine preparations and paraffin sections were studied. Some examination of E.M.C. and of somatic mitosis was made. The paraffin material was fixed in modifications of KARPECHENKO's formalin-chrom-acetic solution and stained with iron haematoxylin. The figures illustrating cytological conditions were drawn from such preparations unless otherwise noted. Miss MABEL L. RUTTLE has given valuable assistance in connection with these cytological investigations.

The complications involved in analysis (both genetic and cytological) when dealing with selfed progenies of eggs and sperms, both of which may possess nuclear modification as a result of irradiation, are obvious. As noted, it was difficult to arrange the variants into classes on the basis of equivalence in external morphology, either total or partial, and it may be that the following classification of the cytological behavior exhibited by certain of these variants has only relative significance. The designations of classes which follow are descriptive of the conditions observed during mitosis or meiosis. Certain of these cytological phenomena in irradiated material have been described by KOERNICKE (5, 6), STEIN (9), PEKAREK (8), and others (cf. HERTWIG 4).

CHROMOSOME NUMBER AND BEHAVIOR NORMAL

Of twenty-nine plants on which cytological data are most extensive, two were entirely normal in external morphology and fully fertile (fig. 2b). They were likewise indistinguishable from the control cytologically, and exhibited 24_{II} at I-M in P.M.C. and E.M.C. The diaphase and metaphase showed close pairing, and there was no evidence of lagging at I, II, or in microcyte number at the pollen tetrad stage. Chromosome conditions of this sort are referred to as "normal." Seven other plants, all variants, were found to be normal cytologically (figs. 3a, 4; and table II, 6, 10). Their fertility was much reduced in crosses with the control, and one of these seven plants was completely sterile. In this first class, then, involving normal cytological conditions, wide variations in character expression and fertility occurred. At one extreme were plants equivalent to the control, and at the other individuals differing from it in all plant characters and more or less completely sterile.

NON-CONJUNCTION

Five plants were included under this heading because, although they sometimes gave counts of 24_{II} at early I-M, they showed loose pairing at diaphase, chromosomes off the equatorial plate at I and II-M, and large numbers of microcytes at the tetrad stage. Since unpaired chromosomes appeared during I, these cases might be included under the next category, that is, "unpaired chromosomes."

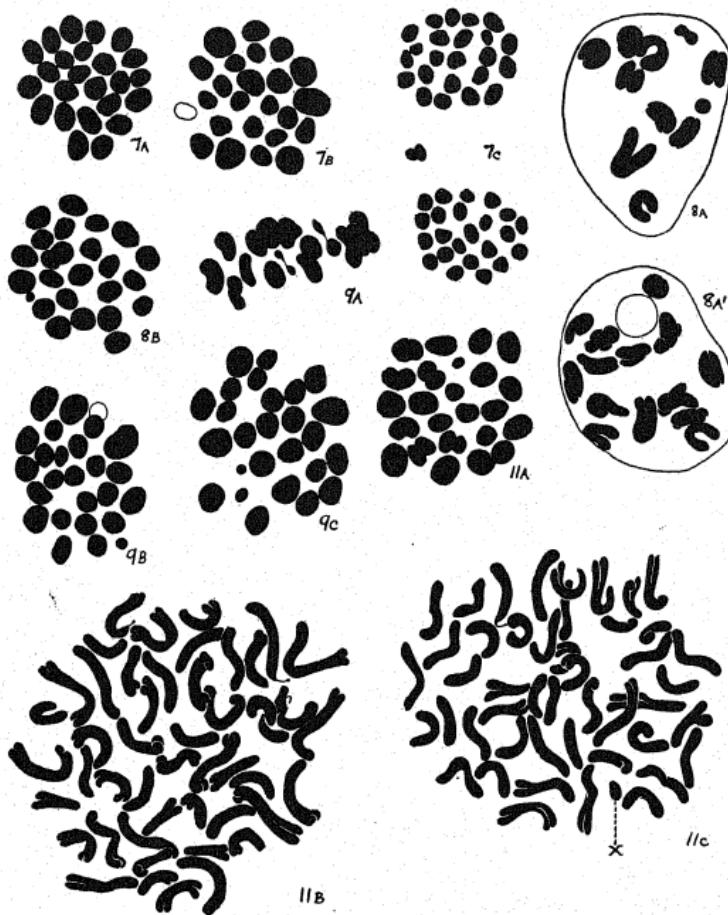
On the other hand, in those instances next described the I-M count was always relatively clear, while here the variation in the number of bivalents and univalents from one M.P.C. or E.M.C. to the next was so great that no final decision could be made as to the extent of conjugation.

The fertility of this group was relatively very low, only two plants giving any seed. In external morphology they were highly abnormal, with the exception of one plant which was a replica of the control but exhibited a greatly reduced fertility. In this plant 24_{II} could at times be counted at I-M; but there were always lagging chromosomes at interkinesis, and at II-M there were usually less than 24 in single plates with from two to five chromosomes in the plasma, and over 40 per cent of microcytes. One striking variant consistently showed from one to three chromosomes off the plate at I-M, and at diaphase 24 units, some very loosely associated. There were over 30 per cent of microcytes and the plant was sterile. Finally there was a highly variant, sterile plant (fig. 4) which apparently possessed a normal somatic chromosome complement, but exhibited a highly variable condition at I-M, the chromosomes being scattered as bivalents and univalents over the spindle and later distributed very irregularly.

UNPAIRED CHROMOSOMES

The chromosome situation in eight plants suggested that they might be the products of relatively simple meiotic aberration following irradiation. Four showed 23_{II} and 1_I, and three showed 24_{II} and 1_I at I-M in P.M.C.; the eighth plant possessed either 22_{II} and 1_I or 23_{II}.

In fig. 7b and c, typical I and II-M conditions in 27154Pr162 are illustrated, together with I-M of the control (a). In chromosome behavior, 27154Pr162 corresponded to monosomic *tabacum* individuals, which previously had occurred in our cultures (2). There were clearly 23 bivalents, and a somewhat smaller, paler chromosome which was, in many cases, off the equatorial plate at early I-M (fig. 7b) and lagged at I-T. In one case at II-M (fig. 7c) 23 chromosomes could be counted in both plates and there was a chromosome dividing in the plasma. In other P.M.C. 23 and 24 were counted at II-M. Approximately 15 per cent of microcytes occurred. Corresponding



FIGS. 7-9, 11.—Fig. 7, I and II-M of P.M.C.: *a*, 27014 (control), I-M 24_{II}; *b*, 27154P162 (table II, no. 13) I-M 23_{II} and 1_I (in outline); *c*, II-M, aceto-carmine preparation, 23 chromosomes in each plate and one in the plasma (after partial division at I-A). Fig. 8, *a*, *a'*, 27154P004 (table II, no. 5) diaphase, P.M.C., 23_{II} plus two chromosome fragments; *b*, 27154P24 (table II, no. 8) I-M, P.M.C., 24_{II} plus one fragment. Fig. 9, 27154P004, P.M.C.: *a*, late I-M, side view, all chromosomes not shown, disjunction beginning, chromosome fragments undergoing early and unequal division; *b*, I-M, 24_{II} plus 1_I and a fragment; *c*, 24_{II} plus two fragments. Fig. 11, *a*, late I-M, E.M.C., 27154P002, 23_{II} plus one chromosome fragment; *b*, somatic metaphase of 014 (control); *c*, same, 27154P002, 47 chromosomes and one chromosome fragment (*x*).

chromosome behavior was found in the remaining three "monosomic" individuals.

In the case of the "trisomic" plants, there was at times some difficulty in deciding whether the chromosome garnitures were not 23_{II} plus 2_I rather than 24_{II} plus 1_I. The II-M showed in most cases 24 and 25, or 24 in each plate with a chromosome in the plasma. More than a single chromosome was at times seen in the plasma at interkinesis. A precocious division of the univalent at I-A, sometimes observed, may account for this variation. On the other hand it may appear after further study that certain of these variant individuals should be included in the previous category of chromosome behavior (non-conjunction). There was little evidence of trivalent occurrence at I-M which corresponds to the condition reported in the case of a known *tabacum* trisomic (1).

None of these eight plants, in most cases presumably the products of non-disjunction occurring subsequent to irradiation, was equivalent in external morphology to any monosomic or trisomic *tabacum* which has appeared in our cultures. In the case of two of the four "monosomic" variants, a strong resemblance to "fluted" *tabacum* (2) was noted. This was also true of a number of the variants in the different X-ray populations, and a certain proportion of the total number of plants of this type should, perhaps, be assigned to induced or spontaneous occurrences of "fluted." The majority, differing markedly from "fluted," undoubtedly involve genic modifications or chromosomal distinctions in addition to the loss of the chromosome which results in the typical change in external morphology.

It would appear, then, that following irradiation of *tabacum*, as in other material also, a considerable increase in the incidence of non-disjunction may be expected to occur, this more simple chromosomal modification being often accompanied by other changes.

CHROMOSOME FRAGMENTATION

Five plants showed evidence of chromosome fragmentation at meiotic stages, and for one of them the presence of a small chromosome in the somatic complex has been demonstrated. Extended studies of chromosome behavior were made in three plants, 27154Poo₂, Poo₄, and Pr68. As will be noted in table II (4, 5, 14), all three

plants were highly variant in external morphology (figs. 2a, 5b). Two showed a reduced and variable fertility, while the third was

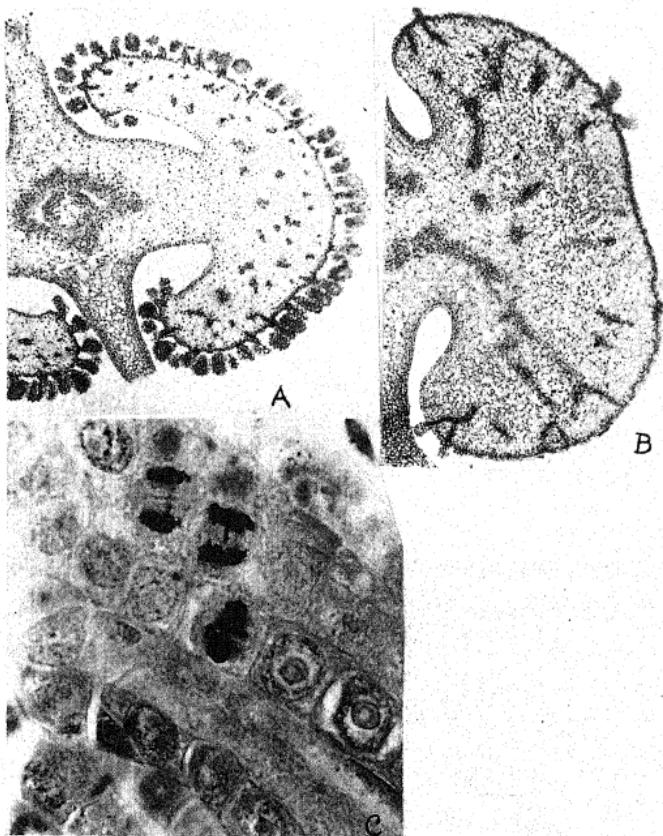


FIG. 10.—*a*, *b*, cross-sections of ovary at anthesis; *a*, 014 (control); *b*, 27154P002 (table II, no. 4) to show absence of ovules; *c*, 27154P004, longitudinal section of root tip to show lagging chromosomes at telophase.

completely sterile. Figs. 8-10 illustrate portions of the cytological evidence obtained.

The presence in these plants of minute chromosomes at diaphase

and I and II-M was clear. In Poo₂ and Pr₆₈ there were 22_n or 23_n plus one such minute unit, in Poo₄ probably 23_n plus two fragments. Of the remaining two plants of this category, the first (P₂₄, fig. 8b) possessed probably 24_n and one chromosome fragment, the second 23_n and one fragment. As the various figures show, the minute units in these five plants were similar in size and form. In meiotic stages some of them were at first seen as appendages attached by delicate strands to an otherwise normal bivalent, and were thus described in an earlier report (3). Imperfect fixation may have been responsible for the earlier decision as to their relation to other chromosomes, although at times and with otherwise successful technique they have again appeared as appendages, particularly at diaphase. At I-M their position either within or without the equatorial plate of bivalents is variable.

Neither at diaphase nor at I-M was there any evidence that the chromosome fragments existed in pairs. Usually they were distributed along with the bivalent partners, and at times divided at II-A (7). They may divide at I, however, and their "halves" complete passage to opposite poles. Such division is almost always precocious and almost complete before the disjunction of surrounding bivalents has been initiated. It also often appeared to be unequal (fig. 9a), so that elements of different size went to opposite poles.

In 27154Poo₄ the diaphases usually showed close pairing (fig. 8a, a'), although a number of P.M.C. were seen in which it was not so precise. There was also some variation in the number of bivalents and univalents at I-M. There were even early I-M in which not less than 45 units could be counted, and, correspondingly, cells at II-M with the products of division of such a "non-reductional" I-M. In such cases the chromosomes at I and II-M were smaller than normal bivalents or products of their disjunction. Such P.M.C., exhibiting an almost complete failure of conjunction, were rare, whereas such conditions as are shown in figs. 8 and 9 were not uncommon. Fig. 9b exhibits 26 units, of which 24 are bivalents, one a chromosome fragment and one a smaller, paler chromosome corresponding in appearance to the univalent in monosomic *tabacum*. It is probable that a second chromosome fragment was present but obscured because of close association with one of the larger bivalents. In fig. 9c there are

again 26 units, but in this case two chromosome fragments appear together with 24_{II}. In the diaphase shown in fig. 8a, α^1 , there are 23_{II} plus two fragments. In other words, there was evidence of variation from one P.M.C. to another in the number of chromosomes present at diaphase and I-M, even after every effort was made under the microscope to obtain evidence which would provide grounds for interpreting these results on a uniform basis.

It was not possible to obtain somatic counts from Poo4. In root tips there was no evidence of degeneration of cell lineages but chromosome lagging was frequently observed. In fig. 10c an earlier and a later telophase are shown, with chromatin strung between the polar accumulations. In one case the cell plate alone is serving to divide this chromatin strand. In some similar cells it did not appear possible that quantitatively equivalent portions were being thus separated to the daughter nuclei. This plant showed variation in degree of fertility from flower to flower. In one bud all P.M.C. and E.M.C. were maturing normally to give well-filled capsules on crossing to the control; other buds contained only degenerating sex cells in various stages of maturation; and still others showed one or two anthers in this condition with the remaining anthers normal. It may be that these abnormalities in somatic mitosis, occurring either in meristematic or archesporial tissue, may be responsible for the observed variation in fertility.

That the chromosome fragments which were so conspicuous at meiotic stages in Poo4 would have been identified in somatic mitoses is indicated by the evidence from Poo2. Fig. 11a shows a late I-M from an E.M.C. of this plant with 23_{II} and one fragment, while fig. 11c illustrates a somatic plate from a root tip which may be compared with the control in fig. 11b. The presence of a strikingly smaller chromosome is clear. Poo2 was highly abnormal in external morphology and completely sterile, degeneration of all the pollen taking place at late tetrad and the placentae being partially or wholly devoid of ovules. This latter point is illustrated in fig. 10b, which may be compared with fig. 10a from the control. In Poo2 the pairing at I-M was more precise than in Poo4, but there was much lagging during both I and II.

The case of 27155Poo1 is outstanding among the plants cytologi-

cal examination of which showed chromosome fragments, in that it closely approximated the control in external morphology and was fertile. There were 24 units at I-M, of which one was minute and chromosome behavior was normal throughout. Evidence as to the cytological condition of the remaining plant in this group is not so extensive. The majority of counts gave 24_{II} and a fragment, but in other cases there seemed certainly to be 23_{II} and a fragment. This plant was highly variant in all characters, and sterile.

Summary

1. Appropriate X-ray dosage applied to flower buds of *Nicotiana tabacum* may be followed by the appearance of large numbers of variant individuals in the selfed progeny.
2. There was evidence that variation can be induced by the irradiation of mature pollen.
3. Apart from the abscission of all smaller flower buds, no visible somatic effect followed the X-ray dosage employed.
4. The X-ray variants exhibited numerous types of modification of all vegetative and floral organs and reduction in fertility to various degrees.
5. Cytologically the variants examined proved (1) to be normal in chromosome number and behavior, (2) to show non-conjunction of one or more pairs of chromosomes, (3) to be the products of non-disjunctional phenomena, and (4) to possess fragmented chromosomes.

UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF.

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A SPECTROPHOTOMETRIC STUDY OF REFLECTION OF LIGHT FROM LEAF SURFACES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 391

CHARLES A. SHULL

(WITH TEN FIGURES)

Introduction

The energy relations of leaves have received too little attention from plant physiologists during the last 25 years. The classical studies of BROWN and ESCOMBE (1) on the interchange of energy between the leaf and its surroundings furnish a very important source of information as to the quantitative income and outgo of energy during the processes of photosynthesis, transpiration, and thermal emissivity. They measured the solar radiation falling upon the leaf with great care, using an excellent type of radiometer for the determinations. By means of the same instrument they evaluated the coefficient of absorption of light by the leaf. The amount of energy utilized by the leaf for internal work, photosynthesis, and evaporation of water was carefully estimated from the actual use of CO_2 and the water vapor given off in transpiration; and the gain and loss of energy during periods of positive and negative thermal emission were calculated from data as to the rate of thermal change between environment and leaf with one degree of difference in temperature between them.

From these various measurements they proceeded to construct a balance sheet of energy income and outgo, which would account for 100 per cent of gain and loss of energy. Although they succeeded in working out such "revenue and expenditure" accounts for the leaf, and balanced them perfectly as good bookkeepers are supposed to do, it must be obvious from careful study of their work that these figures are not as accurate as they appear. One cannot help feeling that the data have been chosen carefully to make a perfect balance. The following example for *Helianthus annuus* gives an idea of these balance sheets, and is adapted from BROWN and ESCOMBE's paper:

| ENERGY INCOME | CALORIES PER SQ. CM. PER MINUTE |
|---|---------------------------------|
| 1. By solar radiation | 0.2746 |
| 2. By negative thermal emissivity | 0.1817 |
| Total income per sq. cm. per minute | 0.4563 |
| ENERGY OUTGO | |
| 1. For photosynthesis | 0.0033 |
| 2. For evaporation of water | 0.3668 |
| Total energy used in internal work | 0.3701 |
| Incident energy transmitted | 0.0862 |
| Total outgo per sq. cm. per minute | 0.4563 |

In another instance the energy changes were calculated on the percentage basis, considering that the total energy receipt is 100 per cent. The disposal of energy received during insolation was accounted for by assigning to each energy-using process its percentage of the total. An example follows:

| ENERGY UTILIZATION | PERCENTAGE |
|-----------------------------------|------------|
| For photosynthesis | 0.66 |
| For transpiration | 48.39 |
| Total internal work | 49.05 |
| Transmitted energy | 31.40 |
| Positive thermal emissivity | 19.55 |
| Total energy used | 100.00 |

One must admire the ingenious methods used by BROWN and ESCOMBE in obtaining the results presented. Their work represents possibly the best quantitative measurements of these physical processes of plant life in physiological literature, but the results as recorded are really too good to be accepted as free from choice of data such as would produce the balance sheet. Such perfect results can be obtained only when some of the determinations are made or approximated by "difference."

The most patent source of error in this work by BROWN and ESCOMBE is one of which they were cognizant, and to which they referred, but neglected because they thought it was a small error. This is the reflection of light from leaf surfaces. They pass over the matter of leaf reflection with the following statement:

The coefficient of absorption of leaf for solar radiation was determined by means of the same instrument (Callendar's self-recording radiometer) in the manner to be described in detail later on. It was taken as the difference between the solar radiant energy falling on the leaf in full sunshine and the amount transmitted, and takes no account of any possible reflection of radiation from the surface of the leaf. With perpendicular incidence the reflected radiation must be very small in amount, but it is well to bear in mind that, strictly speaking, the value of the coefficient of absorption employed includes this reflected portion.

The difficulty in this situation is that, even if we know the value of the reflection factor, and attempt to correct the coefficient of absorption, there is no simple way of correcting the figures in the balance sheet presented, to agree with the changed absorption quantity of energy. It seems therefore that the problems of energy utilization must be restudied, with the reflection factor taken into account.

The utilization of solar energy has recently been summarized diagrammatically from the BROWN and ESCOMBE data by SPOEHR (7), the disposal being accounted for in four fractions. Photosynthesis is represented as using 1 per cent of the total incident energy, while transpiration dissipates 50 per cent of it. About 30 per cent is transmitted, and the other 19 per cent is attributed to reradiation, which should include conduction of energy by the atmospheric gases, removal of energy by convection currents set up in the air from any cause, radiation proper, and light reflection from the surfaces of incidence. No attempt has been made to estimate quantitatively the part that each of these reradiation factors plays in the energy outgo from the leaf; no new data are presented covering any phase of the problem, so that SPOEHR leaves the problem just where BROWN and ESCOMBE left it in their contribution.

The interesting measurements of reflection of energy from leaf surfaces by COBLENTZ (2) were unknown to the writer when, some years ago, he called attention to this error in a brief discussion of transpiration as energy dispersal (6). In this paper it was assumed that the reflection from the leaf might be as much as 10 per cent, in view of the fact that a dead-black surface will reflect 1 per cent of the incident light. At the time this suggestion was made no data had come to the writer's attention upon which an accurate estimation of light reflection from leaves could be based, and the opportunity of making such measurements had not presented itself. In

this paper it will be shown that the estimate of 10 per cent was a very conservative figure.

It is fortunate that COBLENTZ included a few leaves of plants in his studies of the reflecting power of matte surfaces. In making his measurements he used a bolometer, a mirror spectrometer, and a fluorite prism. The angle of incident light in his work was 45° , the light coming from a Nernst glower, and the leaf was placed 6 cm. from the spectrometer slit. The earlier measurements made in 1908 extended from the visible into the infra-red as far as $3300\text{ m}\mu$, and in one case (*Liriodendron*) to $4400\text{ m}\mu$. The curves of reflectivity showed a decrease in reflection of energy with increase in wave length. In general the reflection in the infra-red region is small, but in the near infra-red it may be very considerable, 38 per cent at $950\text{ m}\mu$ for instance.

In his later studies (1912) COBLENTZ measured the reflection of visible solar rays, mostly at $600\text{ m}\mu$, but in a few cases at $540\text{ m}\mu$, in the green region of the spectrum. Because it was felt that these data may not be as well known as they should be, permission was obtained to present them here. With some necessary modification, the data of COBLENTZ's table III and a part of table V are combined in table I.

Table I shows apparently that the general reflection from leaves at 45° incidence is not far from 25 per cent. This value is somewhat higher than is found for reflection normal to the surface. COBLENTZ points out the unexpectedly low results for *Verbascum*, considering its hairy surface. Measurements presented in this paper for the same plant are in complete agreement with COBLENTZ's work, in showing that hairs do not cause reflection of light to any marked extent. The two figures on transmission in table I show that about 20 per cent of the energy passes through the leaf under the conditions of the measurements.

The most recent study of light reflection is by POKROWSKI (4), who used a König-Martens spectrophotometer and measured the reflection from leaves of several species of trees at 90° incidence, comparing the reflection from that of MgO , the albedo of which is about 95 per cent. The species used were *Populus tremula*, *Tilia parvifolia*, *Fraxinus excelsior*, *Ulmus effusa*, *Castanea*, and *Acer*

platanoides. The leaves were tested at 1.5 months old, at various wave lengths from $\lambda = 450$ to $710 \text{ m}\mu$. In the blue region of the spectrum he found 4-5 per cent of reflection, in the green 8-17 per cent, and in the red 4-10 per cent. These values are similar to those in the studies to be reported here for summer verdure. I have found much greater reflection in the case of spring verdure and autumn

TABLE I
REFLECTING POWER OF GREEN LEAVES, 45° INCIDENCE

| PLANT | REMARKS | WAVE LENGTH λ MAXIMUM (PERCENTAGE) | | | |
|-------------------------|--------------|--|-------------|-------------|--------------|
| | | 540 m μ | 600 m μ | 650 m μ | 4400 m μ |
| Trifolium pratense | | 23.9 | 21.3 | | |
| | May 3 | 20.0 | 25.3 | | |
| Syringa vulgaris | June 8 | 23.0 | | | |
| | Transmission | 19.6 | | | |
| Robinia pseudacacia | May 3 | 25.4 | | | |
| | June 8 | 23.9 | | | |
| | Transmission | 20.7 | | | |
| Liriodendron tulipifera | Date? | 21.9 | | 38.0 | 5.6 |
| | May 3 | 28.1 | 27.2 | | |
| | July 29 | 22.0 | | | |
| | October 31 | 47.0 | 48.8 | | |
| Kalmia latifolia | Under side | 23.2 | | | |
| Tilia americana | | 27.7 | | | |
| Ulmus rubra | | 26.9 | | | |
| Quercus rubra | Young leaf | 25.7 | | | |
| Verbascum thapsus | Dark green | 29.3 | | | |
| | | 21.8 | | | |
| | | 24.2 | | | |

coloration, in albino leaves, and in one case of extreme hairiness which gave a white surface (under surface of leaf of *Populus alba*).

POKROWSKI reports some transmission data, showing the transmission of 2-6 per cent at $\lambda = 480 \text{ m}\mu$, 10-29 per cent at $550 \text{ m}\mu$, and 4-10 per cent at $650 \text{ m}\mu$. Using the percentage of reflection as determined, and the transmission data, he has calculated the absorption for leaves of *Tilia parvifolia* and *Fraxinus excelsior*. The lowest absorption shown for *Tilia* is at $550 \text{ m}\mu$, an absorption of 52.7 per cent, and for *Fraxinus* 71 per cent, at the same wave length. He thinks the error in these calculations may not be over 5 per cent, and that they are rather too low than too high.

The interest which had been felt in this problem found no means of expression until April, 1925. At this time a few determinations of the reflection of light from leaf surfaces were made on request by

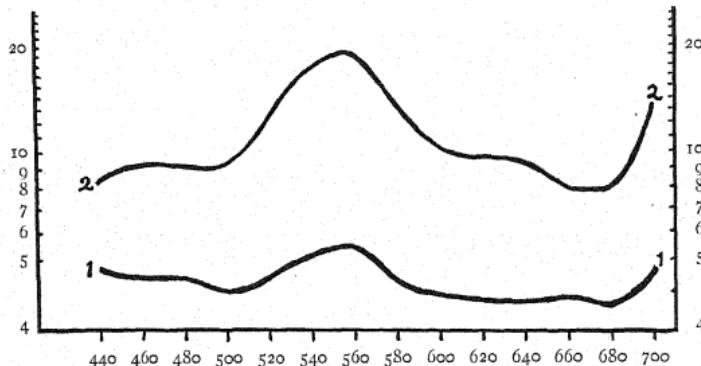


FIG. 1.—Reflection curves for leaves of rhododendron (1), and jonquil (2); note maximum at about $560\text{ m}\mu$, and trough at $680\text{ m}\mu$.

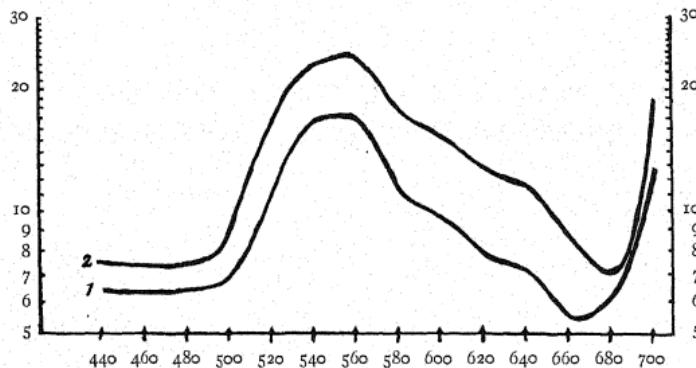


FIG. 2.—Reflection curve for leaves of violet (1), and iris (2); absorption at $660-680\text{ m}\mu$ very marked.

the Keuffel and Esser Company, with their direct reading spectrophotometer or color analyzer. The results were of very great interest, and stimulated the making of the extensive series of measurements here recorded. The plotted curves of reflection made by their

observer, Miss MABEL E. BROWN, are shown in figs. 1 and 2. Fig. 1 shows the reflection curve for leaves of rhododendron and of jonquil, plotted on a logarithmic scale. The leaf of rhododendron, being of a darker green than that of jonquil, reflected a much smaller percentage of the incident light at all wave lengths. In both leaves the maximum reflection is near 555-560 m μ , in the brightest of the green portion of the spectrum, agreeing fairly well with POKROWSKI's absorption minimum at about 550 m μ . Fig. 2 shows the curves of reflection for violet and iris leaves. These show a somewhat different form of curve from those in fig. 1, but still the maximum reflection falls at about the same point in the spectrum. The deep depressions in all of the curves between 660 and 680 m μ lie in the region of the main absorption band of chlorophyll. POKROWSKI reported that the strong absorption maximum of chlorophyll at $\lambda=660$ m μ was not to be seen in his studies. The curves in figs. 1 and 2 certainly show a reflection minimum in that region. In subsequent work this definite depression in the curve at 660-680 m μ was not always seen; however it was frequently observed.

Materials and methods

The leaves of plants of different species vary so much in their superficial physical properties, especially in texture and color, that it is desirable to have a study of the reflection of light from a wide variety of leaves. The leaves of *Verbascum thapsus*, for instance, are so different from those of *Ficus elastica*, that one would expect considerable difference quantitatively in the reflection of light from the leaf surfaces of these plants. Light reflection from vernal vegetation and autumn-colored leaves differs enormously from that from the dark green summer foliage. Even in midsummer foliage, the depth of green color varies widely from species to species, and these differences in color certainly modify the percentage of reflection of incident energy from the surfaces. Leaves discolored by disease or adverse physiological conditions, mildews, infectious or induced chloroses, etc., show increased reflection and decreased absorption of light. Nor do upper and under surfaces of the same leaf reflect equal quantities of incident radiation; in nearly all cases the reflection is considerably greater from the under surface.

On account of this great variability of color and texture, many different kinds of leaves were chosen for this study, but mainly from woody vegetation, trees and shrubs. A partial list of the species used is as follows: *Ulmus americana*, *Betula alba*, *Acer platanoides*, *Syringa vulgaris*, *Psedera quinquefolia*, *Populus alba*, *Prunus pissardi*, *Cersis canadensis*, *Aesculus hippocastanum*, *Sassafras variifolium*, *Castanea dentata*, *Liquidambar styraciflua*, *Catalpa bignonioides*, *Morus rubra*, *Ginkgo biloba*, *Tilia americana*, *Magnolia acuminata*, etc. Among the herbaceous forms, *Zea mays*, *Xanthium italicum*, *Arctium minus*, *Verbascum thapsus*, *Abutilon theophrasti*, *Asclepias syriaca*, etc., may be mentioned. In many of the cases the reflection was measured from both surfaces of the leaves.

The spectrophotometer consists essentially of a prism spectrometer set in front of a spherical box in which two 400 watt lamps are housed. The inside of this spherical box is as white as it can be made. Two blocks of magnesium carbonate serve as reflecting surfaces, and the lamps are so baffled that the light from them does not fall directly upon the reflecting blocks, but is reflected upon them from every possible direction by the curved interior walls of the spherical housing. The reflection is measured perpendicularly to the surface of the blocks of $MgCO_3$, the light coming from these surfaces being directed into the collimator of the spectrometer. After passing through the collimator the light strikes a constant deviation prism, from which it passes to the slit of the telescope. The reflection from the lower block of magnesium carbonate forms the upper half of the field of the telescope, and serves as a comparison field for the reflection from the test object, which is inserted in place of the other $MgCO_3$ block and by its reflection forms the other (lower) half of the field of the telescope. By this arrangement the light from the two spectra are seen side by side, and can be compared as to intensity, just as in other half field instruments, like the MacBeth illuminometer and half field polarimeters.

By means of a wheel, carrying a wave length scale, the constant deviation prism may be adjusted to throw light of any desired wave length into the telescope for comparison. The amount of light coming from the standard block of $MgCO_3$ can be reduced and made equal to that coming from the test object, by means of a rotating sectored double disk. The aperture of the sectors can be changed

while the disks are rotating, by means of a wheel, whose graduated knurled head is scaled to read off the percentage of light transmitted through the revolving sectored disks to the prism. When the two halves of the telescope field show exactly equal illumination at any given wave length, the percentage reading is the percentage of reflection of light at that wave length from the surface of the test object at 90° . The readings are independent of the color vision of the observer, and also independent of the kind of light used to illuminate the specimen under observation. A description of the

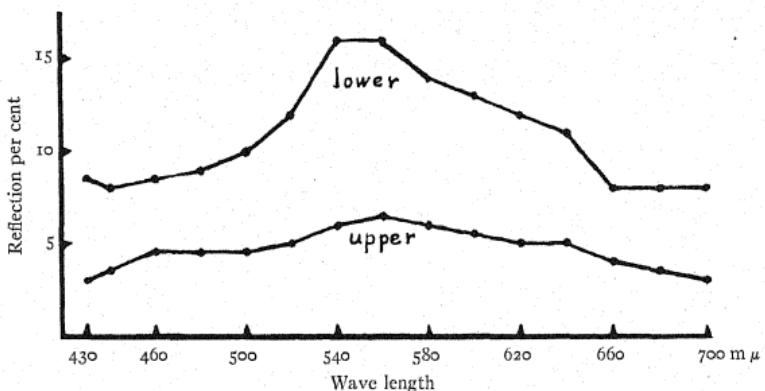


FIG. 3.—Curves of reflection for lilac leaf, comparing upper and under surfaces; curves represent minimum reflection among leaves.

earlier form of this instrument, and directions for its use are given by FERRY (3).

In actual use the upper block of $MgCO_3$ was removed, and the leaf to be measured inserted in its place. The wave length scale on the instrument extends from 430 to 700 μ , and the reflection was usually measured at 430, 440, and at 20 μ intervals across the entire spectrum, closing at 700 μ . The data, in percentage of reflection, are presented in the form of tables and plotted curves.

Results

The reflection data will be shown in several tables, two of which show the general behavior of the late summer dark green foliage, before autumn coloration sets in. Several supplementary tables are

TABLE II
PERCENTAGE OF 90° REFLECTION OF BRIGHT DIFFUSED LIGHT FROM LEAF SURFACES

| SPECIES | SURFACE | WAVE LENGTH TESTED | | | | | | | | | | | | | |
|-----------------------------------|-----------------|--------------------|------|------|------|------|------|------|-------|------|-------|------|------|------|------|
| | | 430 | 440 | 460 | 480 | 500 | 520 | 540 | 560 | 580 | 600 | 620 | 640 | 660 | 680 |
| <i>Ulmus americana</i> | | | | | | | | | | | | | | | |
| Indiana | Upper | 5.0 | 5.5 | 5.5 | 6.0 | 5.0 | 7.5 | 10.0 | 10.0 | 8.0 | 7.0 | 6.0 | 6.0 | 5.5 | 7.0 |
| | Lower | 8.5 | 11.5 | 11.5 | 14.0 | 14.0 | 16.0 | 21.0 | 20.0 | 17.0 | 15.0 | 15.0 | 14.0 | 14.0 | 14.0 |
| Minnesota | Upper | 4.5 | 5.0 | 5.0 | 5.5 | 6.0 | 6.75 | 9.5 | 9.0 | 7.5 | 7.0 | 6.25 | 6.25 | 6.0 | 5.25 |
| | Lower | 5.0 | 5.25 | 6.0 | 6.25 | 7.25 | 9.5 | 11.4 | 11.75 | 10.5 | 10.25 | 9.00 | 8.5 | 8.0 | 6.75 |
| Acer platanoides | | | | | | | | | | | | | | | |
| Indiana | Upper | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.5 | 7.5 | 8.5 | 6.5 | 6.0 | 5.5 | 5.0 | 5.0 | 5.5 |
| | Lower | 10.0 | 10.0 | 11.0 | 11.0 | 12.0 | 16.0 | 19.0 | 20.0 | 16.5 | 16.5 | 14.0 | 14.0 | 12.5 | 15.0 |
| Minnesota | Upper | 3.75 | 3.75 | 3.75 | 3.75 | 4.5 | 5.0 | 6.75 | 6.3 | 5.0 | 4.75 | 4.25 | 4.25 | 4.0 | 3.75 |
| | Lower | 7.0 | 8.25 | 8.75 | 8.75 | 9.5 | 12.0 | 16.0 | 15.5 | 13.5 | 12.25 | 11.0 | 10.0 | 9.0 | 8.75 |
| <i>Syringa vulgaris</i> | Upper | 3.0 | 3.5 | 4.5 | 4.5 | 5.0 | 6.0 | 6.5 | 6.0 | 5.5 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 |
| | Lower | 8.5 | 8.0 | 8.5 | 9.0 | 10.0 | 12.0 | 16.0 | 16.0 | 14.0 | 13.0 | 12.0 | 11.0 | 8.0 | 8.0 |
| <i>Ginkgo biloba</i> | | | | | | | | | | | | | | | |
| | Upper | 6.0 | 5.0 | 6.0 | 6.0 | 6.0 | 9.0 | 14.0 | 15.0 | 12.5 | 10.0 | 10.0 | 9.5 | 8.0 | 11.5 |
| | Lower | 4.5 | 6.5 | 8.5 | 8.5 | 10.0 | 13.0 | 19.0 | 18.0 | 16.0 | 15.0 | 13.0 | 11.5 | 9.5 | 13.0 |
| <i>Magnolia acuminata</i> | | | | | | | | | | | | | | | |
| | Upper | 5.0 | 5.0 | 5.5 | 5.5 | 5.0 | 7.5 | 12.5 | 10.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 |
| | Lower | 18.0 | 20.0 | 20.0 | 17.0 | 18.0 | 21.0 | 25.0 | 23.0 | 22.5 | 22.0 | 19.0 | 17.5 | 17.5 | 19.0 |
| <i>Liquidambar styraciflua</i> | | | | | | | | | | | | | | | |
| | Upper | 6.0 | 6.0 | 6.0 | 6.0 | 6.0 | 9.0 | 11.5 | 12.0 | 9.0 | 8.5 | 8.0 | 7.0 | 6.0 | 8.5 |
| | Lower | 8.5 | 8.5 | 9.5 | 10.0 | 10.5 | 17.0 | 21.0 | 21.5 | 16.0 | 16.0 | 13.5 | 13.5 | 11.5 | 10.5 |
| <i>Catalpa bignonioides</i> | | | | | | | | | | | | | | | |
| | Upper | 3.5 | 3.5 | 3.5 | 3.5 | 4.0 | 6.5 | 9.0 | 11.0 | 9.0 | 7.5 | 7.0 | 6.5 | 5.5 | 5.0 |
| | Lower | 7.0 | 8.0 | 12.0 | 12.0 | 12.0 | 14.0 | 16.5 | 18.0 | 21.0 | 20.0 | 19.0 | 16.0 | 15.5 | 14.0 |
| <i>Castanea dentata</i> | | | | | | | | | | | | | | | |
| | Upper | 5.0 | 5.0 | 5.0 | 5.5 | 5.5 | 7.5 | 9.0 | 9.0 | 7.0 | 6.0 | 6.0 | 6.0 | 5.0 | 6.5 |
| | Lower | 8.5 | 10.0 | 13.0 | 13.0 | 15.0 | 17.0 | 19.0 | 20.5 | 18.0 | 17.0 | 15.5 | 14.5 | 12.5 | 17.0 |
| <i>Sassafras variifolium</i> | | | | | | | | | | | | | | | |
| | Upper | 4.0 | 4.0 | 4.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 7.0 | 6.5 | 6.0 | 5.0 | 5.0 |
| | Lower | 14.0 | 15.0 | 20.0 | 20.0 | 20.0 | 23.0 | 25.0 | 30.0 | 33.0 | 29.5 | 28.0 | 27.0 | 26.5 | 22.0 |
| <i>Cercis canadensis</i> | | | | | | | | | | | | | | | |
| | Upper | 3.5 | 4.0 | 4.0 | 4.5 | 5.0 | 6.0 | 8.0 | 9.5 | 7.0 | 6.5 | 6.0 | 5.5 | 4.5 | 4.0 |
| | Lower | 10.0 | 12.0 | 13.0 | 14.0 | 16.0 | 21.0 | 26.5 | 28.0 | 27.0 | 20.0 | 20.0 | 17.0 | 14.0 | 19.0 |
| <i>Aesculus hippocastanum</i> | | | | | | | | | | | | | | | |
| | Upper | 5.0 | 5.0 | 5.0 | 5.0 | 5.0 | 6.0 | 9.0 | 10.0 | 8.0 | 7.0 | 6.5 | 6.5 | 6.0 | 6.0 |
| <i>Populus alba</i> * | | | | | | | | | | | | | | | |
| | Upper | 6.5 | 7.5 | 9.0 | 10.0 | 12.0 | 14.0 | 19.0 | 20.0 | 16.5 | 13.0 | 12.0 | 12.0 | 10.5 | 10.0 |

* See also table V for the under surface reflection of *P. alba*.

given, to show the influence of age on the percentage of reflection, the effects of hairiness, the reflection from the surface of albino leaves, the effects of anthocyanin development, and autumn coloration. In table II is shown the general reflection situation with respect to the leaves of trees and shrubs, and in fig. 3 is presented a graph showing the lowest reflection found, in the case of *Syringa vulgaris*.

In table II one sees the ordinary range of variability of green working leaves, from about 6.5 per cent in such forms as lilac and the sycamore-leaved maple, to 20 per cent in *Populus alba*. Some of the under surfaces are even lighter in color, as in *Sassafras*, *Magnolia*,

TABLE III
PERCENTAGE REFLECTION FROM LEAVES OF HERBACEOUS PLANTS

| SPECIES | SURFACE | WAVE LENGTHS TESTED (M μ) | | | | | | | | | | | | | | |
|--------------------------|-----------|--------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | | 430 | 440 | 460 | 480 | 500 | 520 | 540 | 560 | 580 | 600 | 620 | 640 | 660 | 680 | 700 |
| <i>Asclepias syriaca</i> | Upper.... | 5.0 | 5.0 | 5.0 | 5.0 | 5.5 | 7.5 | 12.0 | 13.0 | 0.5 | 9.0 | 8.0 | 7.0 | 6.5 | 6.0 | 5.5 |
| | Lower.... | 10.0 | 10.0 | 12.0 | 11.0 | 11.5 | 17.0 | 20.0 | 21.5 | 18.0 | 17.0 | 16.0 | 14.0 | 13.0 | 13.0 | 13.0 |
| <i>Xanthium italicum</i> | Upper.... | 5.5 | 6.0 | 6.0 | 6.0 | 6.0 | 13.0 | 15.0 | 13.0 | 10.0 | 9.0 | 8.0 | 7.5 | 5.0 | 4.5 | 8.5 |
| | Lower.... | 8.5 | 10.0 | 10.0 | 10.0 | 10.0 | 13.0 | 18.0 | 19.0 | 16.0 | 12.5 | 12.0 | 12.0 | 11.0 | 14.5 | |
| <i>Arctium minus</i> | Upper.... | 5.0 | 5.5 | 6.0 | 7.0 | 7.0 | 12.0 | 16.0 | 16.0 | 13.5 | 12.0 | 10.0 | 9.5 | 7.5 | 7.0 | 13.0 |
| | Lower.... | 14.0 | 13.0 | 13.0 | 14.0 | 14.0 | 19.0 | 23.0 | 22.0 | 19.0 | 16.0 | 16.0 | 14.0 | 14.0 | 13.0 | 17.0 |
| <i>Zea mays</i> | Upper.... | 5.5 | 6.0 | 6.5 | 8.0 | 8.0 | 8.0 | 12.0 | 13.0 | 11.5 | 9.0 | 9.0 | 9.0 | 8.0 | 7.0 | 10.0 |
| | Lower.... | 7.0 | 7.0 | 8.0 | 8.0 | 8.0 | 11.0 | 14.0 | 16.0 | 14.0 | 12.0 | 10.0 | 10.0 | 9.0 | 7.5 | 12.0 |

and *Cersis* leaves. In all cases the under surfaces reflect more light than the upper surfaces of the same leaves. This is notably true for the *Sassafras* and *Cersis* leaves. Without exception the maximum reflection falls at 540 or 560 m μ , in the brightest portion of the green region of the spectrum. In the comparison of elm and maple leaves from Indiana and Minnesota, the main difference was that the reflection from the lower surface was less in the Minnesota specimens, indicating that the under surfaces are slightly darker green in Minnesota than in Indiana.

In table III are shown some results with herbaceous plants. These differ in no very striking way from the leaves of woody plants in reflecting power. The depression of the reflection curve in the red is noticeable in *Zea*, *Arctium*, and *Xanthium*, and also in a number of the cases in table II, as in *Ginkgo*, *Castanea*, *Sassafras*, etc.

A few species were found to be developing new leaves as a result

TABLE IV
INFLUENCE OF AGE ON PERCENTAGE OF LIGHT REFLECTION FROM LEAF SURFACES

| SPECIES | AGE | SURFACE | WAVE LENGTH TESTED (M μ) | | | | | | | | | | | | | | |
|--------------------------|------------|------------|-------------------------------|-----|-----|------|------|------|------|------|------|------|------|------|------|------|------|
| | | | 430 | 440 | 460 | 480 | 500 | 520 | 540 | 560 | 580 | 600 | 620 | 640 | 660 | 680 | 700 |
| <i>Tilia americana</i> | Young | Upper..... | 7.5 | 8.0 | 8.0 | 9.0 | 14.0 | 21.0 | 27.0 | 28.0 | 24.5 | 23.0 | 20.0 | 19.0 | 16.0 | 10.0 | 20.0 |
| | Medium | Upper..... | 3.0 | 6.0 | 6.5 | 5.0 | 10.0 | 15.0 | 15.0 | 12.0 | 10.0 | 8.0 | 8.0 | 5.0 | 4.0 | 9.0 | 9.0 |
| | Old | Upper..... | 5.0 | 4.0 | 6.0 | 6.0 | 5.0 | 7.0 | 10.0 | 10.0 | 8.0 | 7.0 | 6.0 | 6.0 | 6.0 | 4.0 | 7.5 |
| <i>Populus deltoides</i> | Young | Upper..... | 3.0 | 3.0 | 5.0 | 6.5 | 6.5 | 12.0 | 21.0 | 22.0 | 21.0 | 16.0 | 14.0 | 12.5 | 9.0 | 7.0 | 6.0 |
| | Old | Upper..... | 4.5 | 5.0 | 5.5 | 6.5 | 8.0 | 10.0 | 14.0 | 16.0 | 12.0 | 11.0 | 9.0 | 8.0 | 7.5 | 7.0 | 6.5 |
| <i>Ficus elastica</i> | Young | Upper..... | 4.0 | 4.0 | 4.0 | 4.0 | 5.0 | 7.0 | 11.0 | 11.0 | 10.0 | 10.0 | 9.5 | 6.5 | 5.5 | 10.0 | 10.0 |
| | Old | Lower..... | 6.0 | 8.5 | 9.5 | 10.0 | 10.0 | 14.0 | 15.0 | 17.0 | 18.0 | 19.0 | 18.5 | 18.0 | 13.0 | 11.0 | 17.0 |
| | Lower..... | Upper..... | 3.0 | 4.0 | 5.0 | 5.5 | 7.5 | 12.0 | 11.5 | 9.0 | 6.5 | 5.5 | 4.5 | 4.5 | 4.5 | 5.5 | 5.5 |
| <i>Cercis canadensis</i> | Young | Upper..... | 3.5 | 4.5 | 5.0 | 5.0 | 5.0 | 6.0 | 9.0 | 9.0 | 7.5 | 6.5 | 5.5 | 5.0 | 4.5 | 3.5 | 4.0 |
| | Old | Upper..... | 3.5 | 4.0 | 4.0 | 4.5 | 5.0 | 6.0 | 8.0 | 9.5 | 7.0 | 6.5 | 6.0 | 5.5 | 4.5 | 5.0 | 4.0 |

of copious rains during the later part of the summer, 1926. Comparative measurements, of upper surfaces mainly, were made of leaves of different age in four species, *Tilia americana*, *Cercis canadensis*, *Populus deltoides*, and *Ficus elastica*. The data are presented in table IV, and the results with *Tilia americana* are shown graphically in fig. 4. In fig. 4 the curve marked "medium" was made

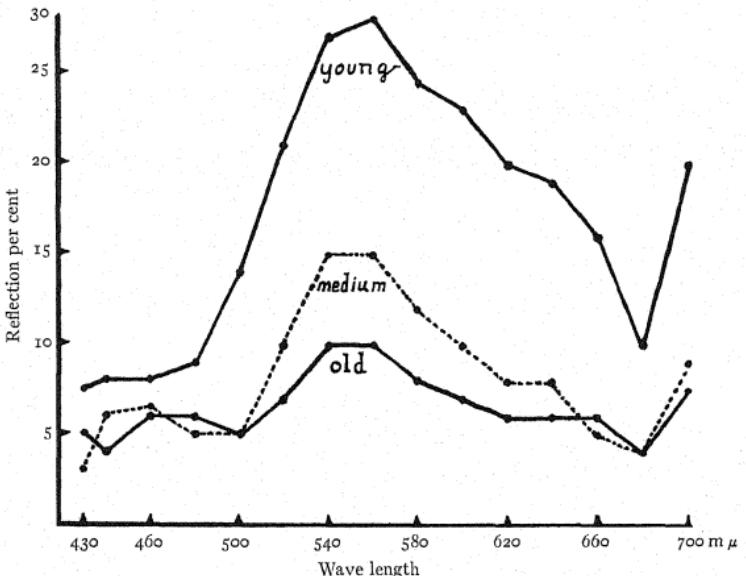


FIG. 4.—Curves of reflection showing effects of aging on leaves of *Tilia americana*; chlorophyll development brings decreased reflection.

from a leaf not much older than the young leaf, possibly only a week or so older. It was intermediate in shade of green to the eye, when seen with the young and old leaf. The old leaf was one which had been functioning all summer. The leaves must change very rapidly from the condition of spring verdure to the full dark green of summer, and yet POKROWSKI (4) shows that leaves at 1.5 months have not yet reached the point of least reflection.

Some attention was given to hairiness, since it was customary formerly to attach some importance to it as an ecological factor.

SAYRE (5) showed some years ago that hairiness had little significance in the process of transpiration. Leaves of *Verbascum*

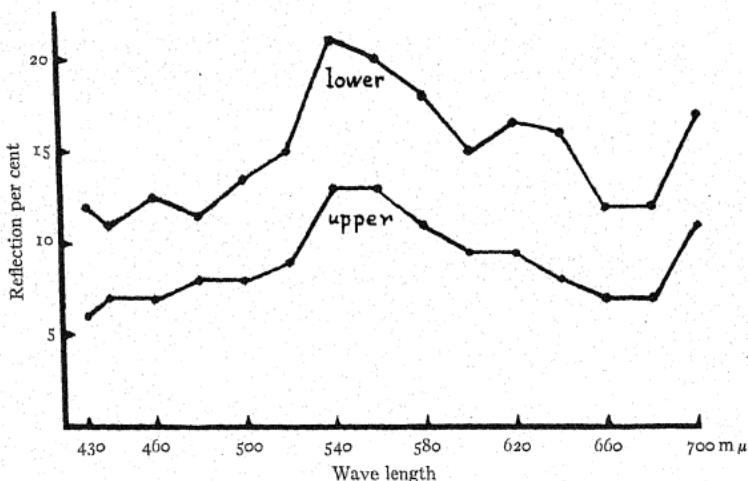


FIG. 5.—Reflection from leaves of *Verbascum thapsus*; in this case hairs have little effect on amount of reflection.

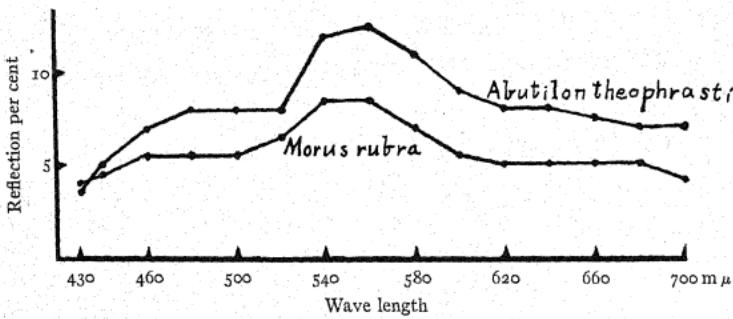


FIG. 6.—Comparison of reflection from upper surface of *Abutilon theophrasti* and *Morus rubra*; neither hairs nor shiny cuticle increase reflection in these cases.

thapsus and *Abutilon theophrasti* were chosen for the presence of hairs, and *Morus rubra* for its very shiny smooth cuticle. The red mulberry leaf has the appearance of having been varnished on its

TABLE V
INFLUENCE OF HAIRINESS ON PERCENTAGE OF LIGHT REFLECTION FROM LEAF SURFACES

| SPECIES | LOCALITY | SURFACE | WAVE LENGTH TESTED (M μ) | | | | | | | | | | | | | | |
|--------------------------|------------|--------------|-------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | | | 430 | 440 | 460 | 480 | 500 | 520 | 540 | 560 | 580 | 600 | 620 | 640 | 660 | 680 | 700 |
| Verbascum thapsus | Indiana | { Upper..... | 6.0 | 7.0 | 7.0 | 8.0 | 8.0 | 9.0 | 13.0 | 13.0 | 9.5 | 9.5 | 8.0 | 7.0 | 7.0 | 11.0 | |
| | | { Lower..... | 12.0 | 11.0 | 12.5 | 11.5 | 13.5 | 15.0 | 21.0 | 20.0 | 18.0 | 15.0 | 16.5 | 16.0 | 12.0 | 12.0 | 17.0 |
| Minnesota | Upper..... | 5.5 | 5.5 | 6.3 | 6.3 | 6.5 | 9.0 | 12.5 | 13.0 | 10.5 | 9.3 | 8.0 | 7.8 | 6.5 | 6.3 | 9.3 | |
| | | { Lower..... | 10.5 | 11.0 | 11.5 | 12.3 | 14.0 | 16.5 | 21.8 | 20.5 | 18.0 | 16.8 | 15.8 | 15.0 | 14.0 | 11.8 | 14.3 |
| Abutilon theo- phasti | Indiana | { Upper..... | 3.5 | 5.0 | 7.0 | 8.0 | 8.0 | 8.0 | 12.0 | 12.5 | 11.0 | 9.0 | 8.0 | 8.0 | 7.5 | 7.0 | 7.0 |
| | | { Lower..... | 10.0 | 11.5 | 13.0 | 13.0 | 13.0 | 17.0 | 19.0 | 17.0 | 15.0 | 14.0 | 13.0 | 12.0 | 10.0 | 10.0 | 11.0 |
| Morus rubra | Indiana | { Upper..... | 4.0 | 4.5 | 5.5 | 5.5 | 6.5 | 8.5 | 8.5 | 7.0 | 5.5 | 5.0 | 5.0 | 5.0 | 5.0 | 4.0 | 4.0 |
| | | { Lower..... | 7.5 | 8.0 | 9.0 | 10.0 | 12.5 | 14.0 | 15.0 | 13.0 | 11.0 | 10.0 | 10.0 | 9.0 | 7.5 | 10.0 | |

upper surface. The reflection from these leaves is shown in table V, and fig. 5 records in graphic form the difference in reflecting power of the upper and lower surfaces of the Indiana specimen of *Verbascum thapsus*. In fig. 6 is shown the reflection from the upper surface of the velvet leaf, *A. theophrasti*, and the red mulberry. The very shiny surface of the latter reflects less light by 4-5 per cent than the hairy surface of *Abutilon*, when measured at 90° to the surface. On the other hand, the reflection from the mullein leaves is no greater than that for many smooth leaves. The hairs must have very little influence on the light relations. SAYRE showed that

TABLE VI
INFLUENCE OF WHITE SURFACES AND ALBINISM ON PERCENTAGE
OF LIGHT REFLECTION

| SPECIES | SURFACE | WAVE LENGTHS TESTED (M μ) | | | | | | | | | | | | | | |
|--------------------------------------|------------|--------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | | 430 | 440 | 460 | 480 | 500 | 520 | 540 | 560 | 580 | 600 | 620 | 640 | 660 | 680 | 700 |
| Populus alba | Upper.... | 6.5 | 7.5 | 9.0 | 10.0 | 12.0 | 14.0 | 19.0 | 20.0 | 16.5 | 13.0 | 12.0 | 12.0 | 10.5 | 10.0 | 8.5 |
| | Lower.... | 50.0 | 50.0 | 50.0 | 50.0 | 50.0 | 51.0 | 50.0 | 52.0 | 53.0 | 51.0 | 52.0 | 51.0 | 50.0 | 51.0 | 50.0 |
| Geranium Green..... | Upper.... | 6.0 | 7.0 | 10.0 | 7.0 | 8.5 | 12.0 | 16.0 | 15.0 | 12.0 | 12.0 | 11.0 | 10.5 | 10.0 | 10.0 | 9.5 |
| | Albino.... | 25.0 | 31.0 | 31.0 | 33.0 | 40.0 | 43.0 | 43.0 | 47.0 | 45.0 | 45.0 | 45.0 | 45.0 | 45.0 | 45.0 | 43.0 |
| Syringa vul- garis Heal-hy.... | Upper.... | 3.0 | 3.5 | 4.5 | 4.5 | 4.5 | 5.0 | 6.0 | 6.5 | 6.0 | 5.5 | 5.0 | 5.0 | 4.0 | 3.5 | 3.0 |
| | Upper.... | 9.0 | 12.5 | 12.5 | 13.0 | 12.5 | 13.0 | 13.5 | 16.0 | 14.0 | 12.5 | 12.0 | 13.0 | 15.0 | 15.0 | 16.0 |

transpiration from these leaves is controlled by stomatal action, just as in non-hairy leaves. COBLENTZ also found that *Verbascum* leaves are much like others in reflecting power, notwithstanding their hairy surface. The leaf of *Verbascum* shows a low reflection in the region of the red absorption band of chlorophyll, as shown in fig. 5. This is not evident, however, in the measurements of *Abutilon* and *Morus* shown in fig. 6. For a case where tomentose hairiness does affect reflection see table VI, the under surface of the leaf of *Populus alba*.

Some leaves have very white surfaces, as has just been mentioned for *P. alba*, and in a few instances we have albino plants, or plants with occasional albino leaves. The common white-edged geranium (Madame Seleroy) bears such leaves, without chlorophyll. Whiteness of the surface may result from the presence of mildew or other

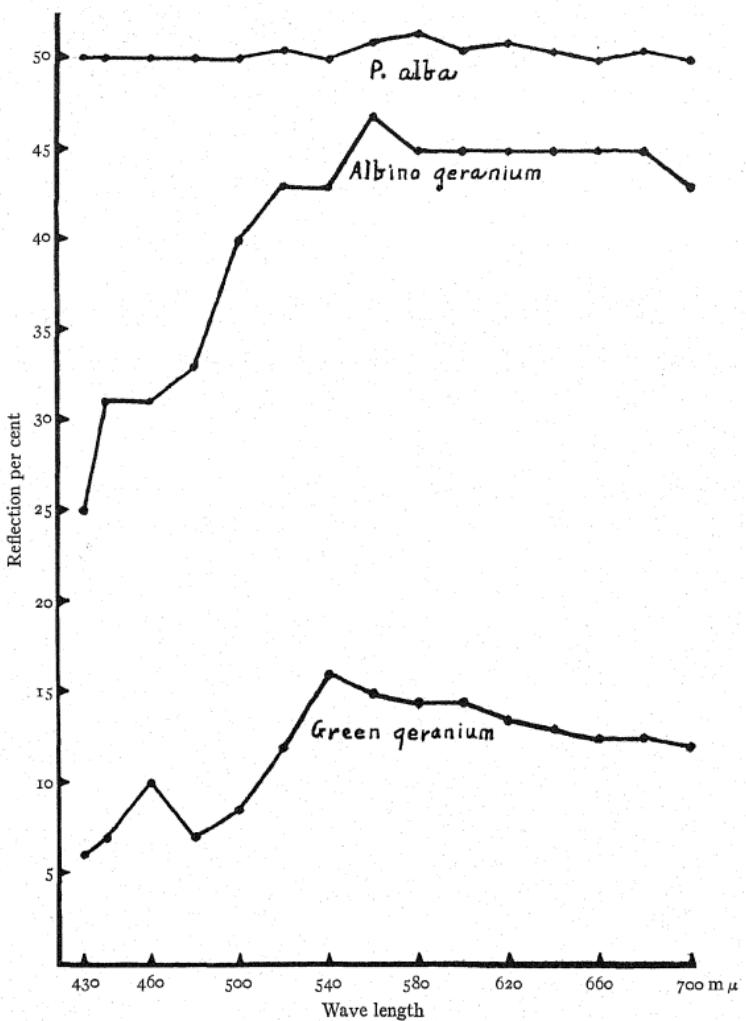


FIG. 7.—Reflection curves for tomentose under surface of *Populus alba* leaf, and for upper surfaces of green and albino leaves of geranium (Madame Seleroy); note uniform reflection of gray color, also smaller reflection in blue region from albino leaves.

fungi, as in the case of the lilac whose leaves at the end of summer are frequently covered with the mycelium of *Micropshaera alni*.

Measurements of both surfaces of the *P. alba* leaves, of green and albino geranium, and the upper surfaces of healthy and mildewed lilacs are presented in table VI.

The extraordinary situation with respect to the lower surface of *P. alba* is just what one would expect on the basis of its color. It looks very white to the eye, but should no doubt be considered as

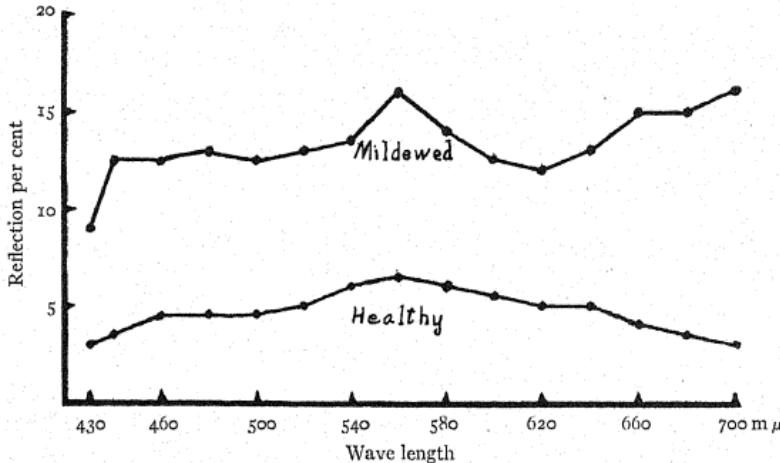


FIG. 8.—Comparison of reflection from healthy and mildewed lilac leaves

gray. The reflection is almost the same across the entire spectrum, usually slightly above 50 per cent. The whiteness is due to the closely matted woolly covering. In fig. 7 is shown graphically the reflection curve for the lower surface of *P. alba*, and a comparison of the green and albino geranium leaf reflection (upper surface in both cases).

The peculiar reflection curve of the albino geranium leaf, low in the blue region as compared with the red portion, is associated with the yellow color of the albino leaves. The yellow color of the leaf is due to the absorption of the blue radiation; and if the reflection were as great from 430 to 560 mμ as elsewhere in the spectrum, one would expect the albino leaf to be almost as white as the under surface of the leaves of *P. alba*.

The effects of the mildewing of lilac can be seen at a glance in fig. 8. The reflection is 2-3 times as great from the diseased as from the healthy leaf. That the light relations are unfavorably affected is not a new idea, but this is probably the first quantitative measure of the effect:

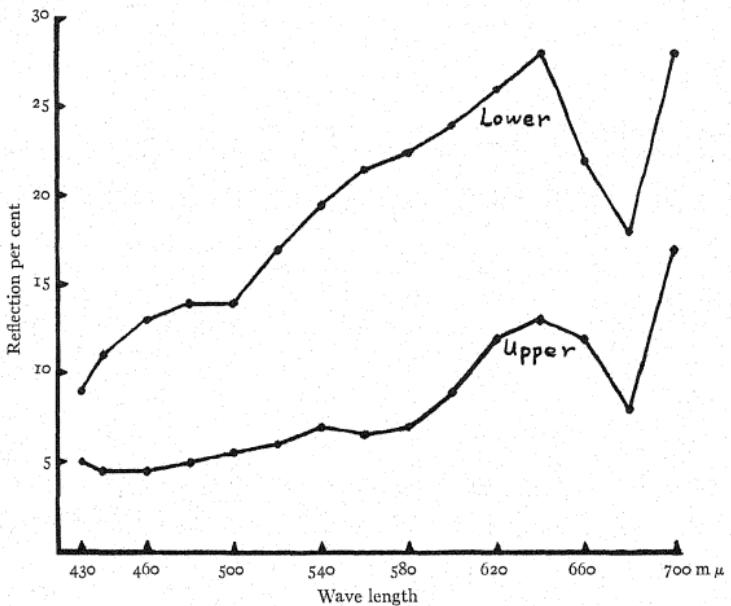


FIG. 9.—Reflection curves for autumn-colored *Psedera quinquefolia* leaves; note reflection peak at 640 and depression at 680 mμ.

The influence of anthocyanin development on reflection was observed in several leaves. The measurements concern *Prunus pissardi*, the lower surface of the leaves of *Acer palmatum atropurpureum*, and the crimson autumn-colored leaves of *Psedera quinquefolia*. The reflection data from these leaves are shown in table VII, and graphs for *Psedera* leaves are given in fig. 9. The maximum reflection is shifted toward the red region of the spectrum, but with a very sharp depression of the curves at 680 mμ. The lower surface is seen to reflect several times as much light as the upper one. A similar

situation is seen in the case of lower surface reflection from the leaf of *Acer palmatum atropurpureum* from 600-700 m μ . The upper surface of this leaf has no anthocyanin, and is in sharp contrast to the lower surface (table VII). The *Prunus pissardi* leaf shows only a slightly higher reflection in the red region than in other portions of the spectrum. Its reflection is lower than that for many other species.

A small number of readings were made of brilliant yellow autumn-colored leaves, particularly of *Betula alba*, and the upper surface of *Populus deltoides*. The data are presented in table VIII,

TABLE VII
INFLUENCE OF ANTHOCYANIN DEVELOPMENT ON PERCENTAGE
OF REFLECTION OF LIGHT FROM LEAVES

| SPECIES | SURFACE | WAVE LENGTHS TESTED (m μ) | | | | | | | | | | | | | | |
|------------------------------------|-----------|--------------------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| | | 430 | 440 | 460 | 480 | 500 | 520 | 540 | 560 | 580 | 600 | 620 | 640 | 660 | 680 | 700 |
| Prunus pissardi | Upper.... | 5.0 | 5.0 | 4.5 | 4.5 | 5.5 | 6.0 | 7.0 | 6.0 | 6.0 | 8.0 | 8.0 | 7.0 | 6.0 | 5.5 | 8.0 |
| <i>Acer palmatum atropurpureum</i> | Upper.... | 4.0 | 5.0 | 5.0 | 5.0 | 5.0 | 5.5 | 6.5 | 7.0 | 6.0 | 5.0 | 5.0 | 5.0 | 4.5 | 5.0 | 5.0 |
| | Lower.... | 6.0 | 7.5 | 8.0 | 8.0 | 7.0 | 8.0 | 8.0 | 8.0 | 9.0 | 10.5 | 12.0 | 14.0 | 14.0 | 11.0 | 13.5 |
| <i>Pseuderis quinquefolia</i> | Upper.... | 5.0 | 4.5 | 4.5 | 5.0 | 5.5 | 6.0 | 7.0 | 6.5 | 7.0 | 9.0 | 12.0 | 13.0 | 12.0 | 8.0 | 17.0 |
| | Lower.... | 9.0 | 11.0 | 13.0 | 14.0 | 14.0 | 17.0 | 19.5 | 21.5 | 22.5 | 24.0 | 26.0 | 28.0 | 22.0 | 18.0 | 28.0 |

and the curves for the *Betula* leaves in fig. 10. These remarkable curves, like the curve for the albino geranium leaf in fig. 7, show low reflection in the blue region, and very high reflection in the red end of the spectrum. The upper surface reflection reaches a maximum of 42 per cent, agreeing rather well with COBLENTZ's forty-five degree readings for *Liriodendron tulipifera* on October 31 (table I), in which he found 47-48 per cent of the light reflected.

The data here presented, taken as a whole, give one a rather general picture of the light reflection situation as a factor in the energy relations of leaves. The significance of the data will be considered briefly in the following section.

Discussion

From the work of COBLENTZ, POKROWSKI, and that here reported, it is evident that a very considerable portion of the visible radiant energy falling upon the leaf is reflected from it. Some of this

reflection is directly from the cuticular surface, and some undoubtedly from reflecting interior surfaces, the light having penetrated, but

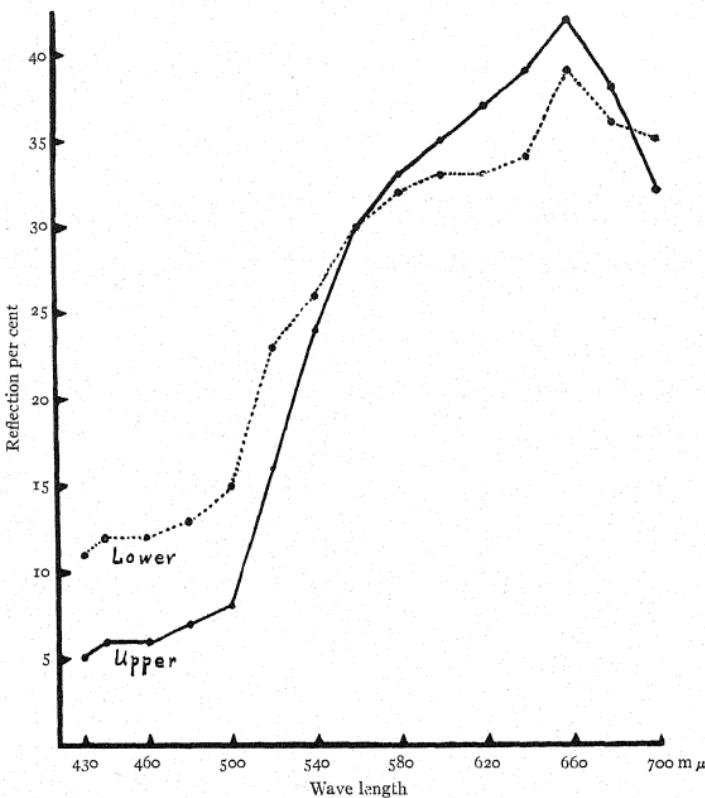


FIG. 10.—Reflection curves for autumn-colored leaves of *Betula alba*; low reflection in blue region is notable in yellow coloration.

being reflected from within. It does not really make much difference whether the reflection is external or internal, for the reflected energy does no internal work, and cannot be transmitted. It must all be subtracted from the total incident energy, just as we subtract

the transmitted energy when we attempt to determine the total absorption of radiation.

The principal value of this work lies in the fact that it shows the magnitude of the reflection factor in the region of visible light. This magnitude is so great that it cannot be neglected in an attempt to establish an energy account for the leaf. It is valuable also in emphasizing the variation of reflection with species. The BROWN and ESCOMBE studies were made only on *Catalpa bignonioides*, which is shown in table I to reflect 6 per cent of the incident light; 11 per cent at maximum in the green. A great many leaves reflect a larger proportion of the light. Data from *Catalpa* alone could hardly be accepted as representative of the entire plant kingdom. The leaf of

TABLE VIII
INFLUENCE OF AUTUMN YELLOW COLOR ON REFLECTION OF
LIGHT FROM LEAVES

| SPECIES | SURFACE | WAVE LENGTHS TESTED (M μ) | | | | | | | | | | | | | | |
|--------------|-----------|--------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| | | 430 | 440 | 460 | 480 | 500 | 520 | 540 | 560 | 580 | 600 | 620 | 640 | 660 | 680 | 700 |
| Populus del- | Upper.... | 8 | 9 | 10 | 11 | 11 | 24 | 33 | 38 | 42 | 40 | 39 | 38 | 38 | 33 | 34 |
| toides | | | | | | | | | | | | | | | | |
| Betula alba | Upper.... | 5 | 6 | 6 | 7 | 8 | 16 | 24 | 30 | 33 | 35 | 37 | 39 | 42 | 38 | 32 |
| | Lower.... | 11 | 12 | 12 | 13 | 15 | 23 | 20 | 30 | 32 | 33 | 33 | 34 | 39 | 36 | 35 |

Catalpa is thin, and many other types of leaves may have a larger absorption coefficient because they are thicker or more deeply pigmented. POKROWSKI found the absorption coefficient to vary from 52 to 95 per cent of the total.

Thickness of the leaf would also become a factor of importance in the thermal emissivity relations. Here again the *Catalpa* leaves are not to be considered as representative, although there are many thin-leaved plants. The absorption coefficient for *Catalpa* given by BROWN and ESCOMBE is probably too large, since reflection was counted in as a part of the absorption. If this is true, then their figures for the internal work must be on the whole too large also.

If there were any simple way in which a correction factor for reflection could be applied to each of the energy changes in internal work, the matter would not be serious, but there is no way in which this can be done. Therefore it seems that the whole question of

energy relations must be reopened and investigated anew, to determine the energy relations for every significant process. This should be done with a wide range of material so that the ordinary limits of variability may be known.

It should be acknowledged at once that, valuable as these spectrophotometric studies are, they are not in themselves sufficient for the revision of the energy relations with respect to reflection. These studies do show that reflection is a large factor and that revision is necessary; but the inadequacy of the spectrophotometer for the ultra-violet and infra-red reflection is obvious. We have measured only the *visible* reflection. While the reflection in the ultra-violet would be small in any event, the infra-red reflection is large. COBLENTZ found 38 per cent reflection at 950 m μ from green leaves of *Liriodendron*. At 4400 m μ it was still 5.6 per cent. To make a proper reflection correction would require a complete study of the spectrum from about 290 m μ to the far limits of the infra red. Absorption should also be studied directly, if possible, with various types of leaves, so that a more accurate balance of the energy changes might be established. Because of the limitations of the spectrophotometer method, it may be necessary at least to supplement it by the use of COBLENTZ's method for exploring the invisible regions of the solar spectrum.

Summary

1. The reflection of light from the surfaces of many kinds of leaves has been measured by means of a direct reading spectrophotometer, the measurements being made normal (that is, at an angle of 90°) to the leaf surface. The incident light, falling upon the leaf from the surface of a spherical housing, strikes the leaf at all angles.

2. The amount of reflection varies with the wave length, the maximum reflection falling usually at 540–560 m μ in green leaves. The value of the reflection in this region runs from 6–8 per cent in the darkest green leaves to 20–25 per cent in the lightest green specimens.

3. Hairiness or smoothness of cuticle does not necessarily mean high reflection. Leaves of *Verbascum thapsus* and *Abutilon theophrasti* show very little more reflection than leaves of non-hairy

plants. The shiny cuticle of *Morus rubra* adds little to normal reflection. In some cases, however, as in the under surface reflection of *Populus alba* and *Magnolia acuminata* leaves, the hairs do increase the reflection.

4. The amount of reflection decreases with the age of the leaf. This is associated with the development of chlorophyll, which increases in amount very rapidly at first, and then more gradually for about two months, until the final depth of green color has been attained. The reflection then remains unchanged until the beginning of chlorophyll destruction at the close of summer.

5. White surfaces reflect almost equally across the spectrum, as seen in the under surface reflection of *Populus alba*; while albino leaves reflect mainly the longer radiations. There is absorption of the blue rays, corresponding to the yellowish color. The reflection from such surfaces is 40-50 per cent of the incident radiation.

6. The presence of mildew or other whitish superficial organisms increases the reflection of light very noticeably. In lilac the presence of *Microsphaera alni* increased the reflection more than 100 per cent.

7. Anthocyanin development is accompanied by a shift in the position of maximum reflection to the longer wave lengths. In *Pseuderis* the maximum reflection occurred at 640 m μ , while the normal maximum for green leaves is 540-560 m μ .

8. Yellow autumn coloration is much more brilliant than red. The reflection of yellow *Betula* leaves reached 42 per cent, with the maximum at 660 m μ . A leaf of *Populus deltoides*, less completely yellowed, reflected as much light, but the maximum reflection was in the yellow, at 580 m μ , instead of in the red.

9. In a considerable number of cases there is a depression of the reflection curve at 680 m μ . This obviously corresponds to the maximum absorption band of chlorophyll. The fact that it is evident is an argument for reflection from internal surfaces, in part.

10. The data presented are valuable in connection with the problem of income and outgo of energy in the leaf processes; but they must be supplemented with measurements in the invisible regions of the spectrum, particularly in the infra-red region, before a complete accounting of the energy utilization can be rendered.

It is a pleasure to record indebtedness to the Keuffel and Esser

Company for their interest in this work, and also to Professor ERVIN S. FERRY, of the Department of Physics, Purdue University, in whose laboratory many of these measurements were made in September, 1926. Acknowledgment is likewise made to Professor CLYDE H. BAILEY, of the Department of Agricultural Biochemistry, University of Minnesota, in whose laboratory some comparative tests were run in October, 1926, with a more recent model of the spectrophotometer. A few measurements of highly colored autumn yellow leaves were made at Minnesota also. The spirit of cooperation and helpfulness shown in these laboratories is gratefully recorded.

UNIVERSITY OF CHICAGO

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MEIOTIC PHENOMENA IN CERTAIN GRAMINEAE¹

I. FESTUCEAE, AVENEAE, AGROSTIDEAE, CHLORIDEAE, AND PHALARIDEAE

GEORGE L. CHURCH

(WITH PLATES XXV-XXVII)

Introduction

The statement of CHARLES DARWIN that the facts of variation under hybridization did not seem to him opposed to the belief that "species aboriginally existed as varieties" is indeed prophetic, in the light of the research of the last two decades. ROSENBERG's work on a natural *Drosera* hybrid (43), involving a cross between parents of different chromosome complements, clearly demonstrated the correlation between cytological abnormalities of the pollen mother cells during the maturation divisions and the sterile pollen resulting from such crossing of species. Similar and even more involved cytological conditions have been found to be widespread among the species of many taxonomically variable plant groups. The researches on *Hieracium* (ROSENBERG 44), *Rosa* (TÄCKHOLM 47), *Rubus* and *Crataegus* (LONGLEY 32, 33), and *Viola* (CLAUSEN 6) are outstanding examples. LOTSY (37) states:

While DE VRIES in his mutation theory predicted that the cause of the polymorphy in *Oenothera*, *Draba* and *Viola*, as well as the nebulae of the older systematicists in *Rubus*, *Hieracium*, *Rosa* and *Salix*, would prove to be due to mutation, we now know that in all these cases hybridization is responsible for by far the greater part of that polymorphy.

In view of the importance of hybridization in the multiplication of species in a host of large genera and families, a cytological investigation of the pollen mother cells of typical representatives of such a large and widespread family as the Gramineae has been considered capable of yielding further significant evidence in this direction.

The hybrid origin of many of our cereals has already been demonstrated by careful genetical and cytological research. Very complete

¹ Contribution from the Laboratories of Plant Morphology, Harvard University.

work has been done on *Triticum* by SAX (45). The wheats fall into three definite polyploid groups, diploid, tetraploid, and hexaploid. SAX considers that the differential mating of chromosomes in crosses between these groups indicates a probable hybrid ancestry for the polyploid members. PERCIVAL (41) notes the extreme antiquity of all the *Triticum* groups, certainly necessitating a very remote period of hybridization. His work on *Aegilops* leads him to the opinion that in this genus may be found one of the ancestors of the "vulgare" or hexaploid wheats.

The same polyploid groups occur in *Avena* as occur in *Triticum*. HUSKINS (24) finds that our commonest oat species, *A. sativa*, often gives rise to so-called "fatuoid" types with aberrant chromosome complements:

The ordinary practically awnless type of *A. sativa* is considered as being the resultant of diverse factors present in its constitution on account of its polyploid and probable hybrid origin.

COLLINS (8) maintains that the cultivated *Zea mays* is of hybrid origin, involving a cross between annual Teosinte (*Euchlaena mexicana*) and some species of *Andropogon*. COLLINS' supposition has been substantiated by the cytological researches of KUWADA (31) and LONGLEY (34). VINALL (52) infers a hybrid lineage for our cultivated sorghums as a result of cross experiments with wild sorghum and Johnson grass.

There are many indications that hybrid grasses exist in addition to the cultivated forms. BREMER (4) considers that the polyploidy of many members of the Andropogoneae allied to *Saccharum* is due to hybridization. EVANS (13) has made chromosome counts in species of *Lolium* and *Festuca* and notes indications of polyploid series similar to those in the cereals. TURESSON (51), in a taxonomic study of the variable *Festuca ovina*, notes normal sexual, facultative and wholly viviparous types in Sweden in a series progressing northward. He suggests that the sterile types are a result of crossing, and compares them with the apomictic *Hieracium* forms in which hybridization has been firmly substantiated. That the sterile pollen and extreme variability of the dichotomum section of the genus *Panicum* indicate hybridity has been accepted very generally. JEFFREY (26) notes a considerable percentage of bad pollen in the common *Alop-*

ecurus pratensis. COCKAYNE (LOTSY 38) lists many systematically recognized hybrid grasses growing in New Zealand; hence the need of cytological data concerning some of our common grasses is evident.

Materials and methods

The grasses examined in this research were collected from various park areas and reservations about greater Boston. Some few exotic species were obtained in the Botanic Gardens of Harvard University and in waste land bordering railroads. Collecting was limited to the early afternoon hours of warm days during the season when the pollen is maturing. A special effort was made to collect those species that revealed bad pollen upon examination of material from the collecting season of the previous year. Carnoy's fluid was employed as a killing and fixing agent, and was pumped in with a field exhaust pump to insure rapid penetration.

The nature of the open panicle type of inflorescence found in many grasses would naturally make the imbedding of a quantity of spikelets exceedingly difficult if there were not some means of concentrating the latter in a compact group. The "glycerin-jelly" method for imbedding small objects in mass as developed in the laboratories of plant morphology of Harvard by Professor E. C. JEFFREY has been employed with complete success in this work. The material was imbedded in nitrocellulose, and 5μ sections were made on a Jung-Thoma sliding microtome. The usual Haidenhain's iron-alum haematoxylin stain was employed, with a weak solution of eosin in 30 per cent alcohol for cytoplasmic contrast. Preparations were studied with a Zeiss 1.5 mm. apochromatic or a Bausch and Lomb 97 \times oil immersion objective and a no. 12 compensating ocular. Drawings were made by the aid of a Bausch and Lomb camera lucida and a stage micrometer to calculate the magnifications.

Cytology of species

The sequence of the tribes and the nomenclature follow the treatment of HITCHCOCK (21). Revisions appearing subsequent to his publication and appearing in the files of the Gray Herbarium of Harvard University are indicated. The present article deals with species studied in the Festuceae, Aveneae, Agostideae, Chlorideae,

and Phalarideae. A second article will deal with species in the Paniceae and Andropogoneae.

FESTUCEAE

Festuca ovina L., Sheep Fescue (pollen 10-20 per cent imperfect).—Chromosome counts of diakinesis stages and heterotypic equatorial plates reveal the octoploid number of twenty-eight bivalents (fig. 8). Many of the divisions are quite regular, resulting in normal tetrads and pollen; however, not infrequently unpaired mates are found in diakinesis. These chromosomes may be extruded as univalents into the cytoplasm during heterotypic metaphase stages, the latter often presenting lagging bivalents as well (fig. 6). The following anaphase in even the last stages shows lagging bivalents (fig. 7). These laggards persist during the diad stage (two cells in a common gelatinous matrix at the end of the heterotypic division), and during the homeotypic division, with the resultant production of aberrant nuclei (polycary) in the tetrads and consequent abortive pollen grains.

Festuca rubra L., Red Fescue (pollen about 25 per cent imperfect).—Diakinesis shows twenty-one ringed bivalents (fig. 9). Equatorial plates also reveal the hexaploid number. Cytomyxis may occur in the prophase stages, often resulting in the persistence of chromatin in the cytoplasm during subsequent divisions. Chromosomes may often become stranded in adjacent mother cells during diakinesis (fig. 9), and give rise to the extrusions found in the following reduction division (fig. 10). The latter figure also shows the common tendency of some bivalents to separate before others and become stretched over the spindle. Such irregular disjoining of the bivalents accounts for the laggards in the early anaphase stages. The homeotypic divisions are quite normal, and extruded chromatin seems to be dissipated by the time the tetrad stage is reached.

Festuca duriuscula L., Hard Fescue; a European introduction collected in the Harvard Botanic Garden plots (pollen 60-70 per cent imperfect).—This species is also hexaploid, as shown by the twenty-one bivalents at the equatorial plate (fig. 11). Laggards and extruded chromosomes are common in the heterotypic metaphase (fig. 1) and anaphase (fig. 2). Frequently one or two bivalent pairs separate in advance of the others, giving the irregular appearance noted

in *F. rubra*. Extruded chromatin, obviously in the path of obliterated spindle fibers, often appears even when the mother cell is nearly split at the equator to form the diad stage (fig. 4). Chromatin has been observed to bridge even the gap in this split. The homeotypic divisions are normal (fig. 5), but extrusions from the heterotypics may persist and give rise to polycaric tetrads. The phenomenon of cytomyxis is not uncommon at the early interkinesis stage (fig. 3).

Dactylis glomerata L., Cocksfoot or Orchard Grass; naturalized from Europe (pollen 40-50 per cent imperfect).—The tetraploid complement of fourteen bivalents published by DAVIES (10) is confirmed by examination of this material. Although the divisions are not generally characterized by lagging or extruded chromatin, the phenomenon of non-pairing is rather conspicuous. Late diakinesis, characterized by a large nucleus and a much vacuolated nucleolus, often shows twenty-eight univalents. These univalents pair tardily just before the spindle appears, but loosely associated pairs may be seen in the early heterotypic metaphase. Diakinesis stages of part bivalent and part univalent chromosomes may also be observed. Polar views showing unpaired mates at the plate are of frequent occurrence. The large percentage of imperfect pollen in the absence of polycary is striking.

AVENEAE

Avena sativa L., common oats; an escape from cultivation and conforming closely to *A. sativa* L. var. *mutica* Al. according to KOERNICKE (in CARLETON 5) (pollen normal).—The twenty-one bivalents at the equatorial plate (fig. 13) mark the strain as hexaploid, corresponding to all the types of *A. sativa* investigated (HUSKINS 24). Normal divisions and tetrads are found, but many outstanding irregularities make their appearance. Lagging bivalents in the form of rings appear in both early (fig. 12) and late (fig. 14) heterotypic metaphases. Likewise lagging univalents are as frequent in the corresponding stages of the anaphase (figs. 15, 16). Occasionally an extreme case of lagging and extrusion may be noted in the telophase (fig. 17). The chromosomes are quite large, and often the homeotypic split can be seen anticipated as a notched groove in the disjoined univalents. Laggards again appear in both stages of the homeotypic division (figs. 18, 19). The homeotypic telophase (fig.

20) is marked by extrusions closely aggregated about the nuclei, which result in a high degree of polycary in the tetrads (fig. 21). The normal pollen is in surprising contrast to such conditions in the maturation divisions.

AGROSTIDEAE

Ammophila breviligulata Fern. The species of the eastern United States coast has been shown (FERNALD 15) to be quite distinct from the European *A. arenaria* (L.) Link in its much larger spikelike panicles and truncated ligule (pollen 40-50 per cent shriveled).—The tetraploid number of fourteen bivalents may be counted in the diakinesis stage, or in polar view of the equatorial plate. Cytomyxis is observed at diakinesis in some instances. Mother cells in a complete state of collapse may be found as a result of excessive chromatin loss. These appear in the same anther sac with normal mother cells. Laggards are not uncommon in the heterotypic anaphase, all other divisions being quite normal. Extrusions resulting from lagging and probably also from cytomyxis may persist in the cytoplasm of the mother cells throughout the second division and give rise to polycaric tetrads.

Alopecurus geniculatus L. var. *aristulatus* Torr. According to GRAY's manual this variety is introduced from Europe in the eastern United States, but blends into the indigenous *A. geniculatus* L. in the western states (pollen 30-40 per cent imperfect).—This is one of the few grasses found in this research with the diploid complement of seven bivalents. The chromosomes are comparatively large, and appear clearly as rings in diakinesis, in which stage they show also a decided tetrad structure. Cytomyxis occurs at prophase, diakinesis, and occasionally at the early heterotypic metaphase. In the last two stages whole chromosome pairs are seen stranded between two mother cells. Laggards in the early heterotypic metaphase usually persist as such in the late metaphase. The early anaphase is frequently marked by a partly split bivalent that stretches along the spindle in advance of the other pairs. One or more tardily splitting bivalents may accordingly be seen in the late anaphase, and extruded into the cytoplasm in the telophase. Occasionally lagging chromosomes appear in both the homeotypic metaphase and anaphase.

Laggards from either division may be extruded and cause aberrant nuclei to form in the tetrads (polycary).

Alopecurus pratensis L., Foxtail Grass; naturalized from Europe (pollen 20-30 per cent imperfect).—Diakinesis reveals fourteen ringed bivalents, making this a tetraploid species. This confirms the number reported by MARCHAL (39). Cytomyxis is frequent in occurrence in the spireme and diakinesis stages. Excessive chromatin loss may result in the disintegration of the mother cell involved, as noted in the case of *Ammophila* and *Festuca duriuscula*. Bivalents at the plate but with half of the ring stranded in the cytoplasm of a closely adjacent cell are noted occasionally in the first division. Unpaired mates may be found in diakinesis which lag as univalents on the heterotypic spindle. Such univalents are either extruded into the cytoplasm or are included in the telophase nuclei, remaining at the poles during the metaphase. The nucleolus has been seen stranded on the spindle in a few instances. The heterotypic anaphase is often marked by laggards which result in a prominent streak of chromatin stretched between the telophase nuclei similar to the condition figured for *Festuca duriuscula* (fig. 4). The extrusions from the heterotypic division usually persist throughout the homeotypic, which may less frequently present laggards and extrusions. Polycary is not uncommon.

CHLORIDEAE

Spartina michauxiana Hitch. (pollen 30-40 per cent imperfect).—This is a tetraploid species, the diakinesis clearly showing fourteen bivalents (fig. 29). The heterotypic division (figs. 30, 31) proceeds in quite regular fashion, as does the homeotypic. Only rarely does a chromosome become extruded during the heterotypic anaphase. All stages seem to be marked by cytoplasmic chromatin, which persists frequently and causes the appearance of polycaric, vacuolated tetrads (fig. 32). So far as the investigated material is concerned, the cytoplasmic chromatin seems to owe its presence in part to chromatin interchange at cytomyxis which may occur in the prophase stages.

Spartina alterniflora Loisel. var. *glabra* (Muhl.) Fern.² (pollen

² See FERNALD (14) for revision of species names.

40-50 per cent imperfect).—Careful examination of the diakinesis stage reveals fourteen bivalents (some loosely paired) and fourteen univalents, the somatic total making the species hexaploid (fig. 22). It will be noted that at even the late stage of the diakinesis figured, as is evidenced by the vacuolated nucleolus, the univalents still retain remnant, threadlike connections; hence they are not clearly delineated in earlier stages because of their persistence in the spireme. These univalents are extruded freely into the often vacuolated cytoplasm during the heterotypic metaphase, some few remaining on the spindle (fig. 23). Occasionally some of the univalents may split at the plate with the bivalents, as is evidenced by the polar view of the metaphase (fig. 24). It is exceptional to find apparently all of the univalents on the spindle, however, as is figured in this diagram. Cytomyctic strands, facilitating the passage of chromatin between mother cells, may also be seen during the heterotypic division (fig. 25). The bivalents move quite regularly to the poles in the anaphase, the univalents or split univalents, if there are any persistent, being distributed at random (fig. 26). This figure also shows many of the extruded univalents coalescing into larger masses preparatory to disintegration in the cytoplasm. There is no splitting of the univalents included in the nuclei during the homeotypic division (fig. 28), as is evidenced by a comparison of a polar view of the homeotypic metaphase with that of the heterotypic (fig. 24). These univalents still lag on the spindle, however, the bivalents proceeding in normal fashion (fig. 27). Extruded chromatin still present in the cytoplasm at this stage causes the not infrequent appearance of polycary in the resultant tetrads.

PHALARIDEAE

Phalaris canariensis L., Canary Grass; annual, advanced from Europe (pollen mostly perfect).—The chromosomes of this species are rather large and clearly show their tetrad composition. Only six bivalents appear in the easily counted metaphase plates seen in polar view. Cytomyxis is observed in the spireme stages, the mother cells so involved usually disintegrating as a result, as is shown by their occurrence in the latter condition in anthers with normal mother cells. The heterotypic metaphase is quite regular. The fol-

lowing anaphase, however, is often marked by a very tardily splitting chromosome pair. Homeotypic divisions are quite regular, and there is no polycary; nevertheless a small percentage of shriveled pollen may be observed.

Phalaris arundinacea L., Reed Canary Grass; a native lowland meadow species (pollen perfect).—Diakinesis counts show unmistakably seven bivalents, often paired as rings. The size of the chromosomes again brings to light the tetrad structure of the pairs, especially in the heterotypic metaphase preparatory to splitting. Divisions, tetrads, and pollen are in every respect normal.

Phalaris arundinacea L. var. *picta* L., Ribbon Grass; a native of Eurasia and commonly cultivated (pollen 30-40 per cent imperfect).—Diakinesis and heterotypic metaphase plates show fourteen bivalents, the tetraploid complement, one or two bivalents appearing separated as univalents. These univalents consistently lag on the spindle, remain at the poles, or become extruded during the heterotypic metaphase. Not infrequently a bivalent may behave in the same manner. An early anaphase stage shows such laggards still at the poles. The extruded chromosomes seem to disintegrate universally. Laggards at the poles become included in the chromosome groups and subsequently resulting nuclei of the late anaphase. Laggards have been noted in the telophase, but again apparently are always dispersed in the cytoplasm before subsequent divisions are initiated. Homeotypic divisions and tetrads are quite normal in appearance. The phenomenon of cytomyxis is widespread in the species, evidently causing much mother cell disintegration in the spireme stage. Parts of chromosomes may be stranded in adjacent mother cells during the heterotypic metaphase, as a result of cytomyxis in the diakinesis. Chromatin exchange not infrequently occurs again at interkinesis. Cytoplasmic strands without interchanging chromatin may be seen connecting the majority of the mother cells. Polycary has not been observed.

Discussion

POLYPLOIDY

A comparison of the chromosome numbers of the various species and genera of grasses investigated in this research discloses a decided

majority of the cases falling into the groups of the higher multiples of the basic haploid number, which for the most part is seven. The thirteen species reported in this article show three diploids, five tetraploids, four hexaploids, and one octoploid. Grouping these with the species of cereals and allied forms already reported (table I), we find the same correlation in respect to the polyplloid tendency. Polypliody is now known to be of quite general occurrence, especially in the larger and consequently variable families and genera. Its extent is coming to be realized as increasingly widespread in the light of rapidly accumulating data.

The great family Compositae has received attention in several genera. ROSENBERG (44) has demonstrated diploids, triploids, and tetraploids in *Hieracium*. The same series with the added category of hexaploids is seen in *Erigeron* (HOLMGREN 23). *Lactuca* and *Crepis* are complicated by the presence of several series of haploid numbers, but the former reaches a variety which is certainly hexaploid (ISHIKAWA 25), and the latter attains octoploidy (COLLINS and MANN 9). A now classic series is shown by the work of TAHARA (48) on *Chrysanthemum*, which presents a series running to decaploidy. The *Chrysanthemum* series is far outrivaled in degree of polypliody by that of *Senecio* (AFZELIUS 2), which has been shown to have not only decaploids and dodecaploids, but even a species with ninety as the haploid number, the haploid base being five!

The Rosaceae presents further striking examples of the occurrence of polypliody. TÄCKHOLM's (47) well known work on *Rosa* has demonstrated large polypliod series in all the groups through pentaploidy as well as a few hexaploids and octoploids. A similar exhaustive work on *Rubus* (LONGLEY 32) has revealed many types falling in all classes of nuclear multiples through hexaploidy. LONGLEY's work on *Crataegus* (33) has shown a considerable amount of triploidy and tetraploidy. *Fragaria* (LONGLEY 35) contains hexaploids and octoploids.

A brief survey of the occurrence of polypliody in two large families in addition to the Gramineae has furnished ample evidence of the importance of the phenomena in considering the numbers and diversity of these groups. The cause of polypliody, especially in the Compositae and the Rosaceae, has been shown by JEFFREY (27) to

be due obviously to hybridization, particularly when the concomitant appearance of chromosome misbehavior, polycary and poly-

TABLE I
EXTENT OF POLYPLOIDY IN THE GRAMINEAE

| | DIPLOID | TETRAPLOID | HEXAPOLOID | OCTOPLOID |
|--|---------|------------|------------|-----------|
| <i>Festuca</i> | | | | |
| <i>ovina</i> *..... | | | | 28 |
| <i>rubra</i> *..... | | | 21 | |
| <i>duriuscula</i> *..... | | | 21 | |
| <i>elatior</i> (Evans 13)..... | | | 21 | |
| var. <i>arundinacea</i> | | | | |
| var. <i>pratensis</i> | 7 | | | |
| <i>Phragmites</i> | | | | |
| <i>communis</i> (Tischler 50)..... | | 18 | | |
| <i>Dactylis</i> | | | | |
| <i>glomerata</i> * (also Davies 10)..... | | 14 | | |
| <i>Agropyrum</i> | | | | |
| <i>repens</i> (Stolze 46)..... | | | 21 | |
| <i>Triticum</i> (cf. complete list in Gaiser 17) | 7 | 14 | 21 | |
| <i>Aegilops</i> (cf. complete list in Gaiser 17) | 7 | 14 | | |
| <i>Hordeum</i> (cf. complete list in Gaiser 17)..... | | | | |
| <i>Secale</i> (cf. complete list in Gaiser 17)..... | 7 | | | |
| <i>Lochium</i> | | | | |
| <i>perenne</i> (Evans 13)..... | 7 | | | |
| <i>Avena</i> | | | | |
| (Gaiser 17)..... | 7 | 14 | 21* | |
| <i>Arrhenatherum</i> | | | | |
| <i>elatum</i> (Aase and Powers 1)..... | | 14 | | |
| <i>Ammophila</i> | | | | |
| <i>breviligulata</i> *..... | | 14 | | |
| <i>Alopecurus</i> | | | | |
| <i>pratensis</i> * (also Marchal 39)..... | | 14 | | |
| <i>geniculatus</i> | | | | |
| var. <i>aristulatus</i> *..... | 7 | | | |
| <i>Spartina</i> | | | | |
| <i>michauxiana</i> *..... | | | 14 | |
| <i>alterniflora</i> | | | | |
| var. <i>glabra</i> *..... | | | | |
| <i>Anthoxanthum</i> | | | | |
| <i>odoratum</i> (Marchal 39)..... | 8 | | | |
| <i>Phalaris</i> | | | | |
| <i>canariensis</i> *..... | 6 | | | |
| <i>arundinacea</i> *..... | 7 | | | |
| var. <i>picta</i> *..... | | 14 | | |

* Species investigated in this research. Numbers refer to the haploid complement.

spory, are taken into account. The investigators of the several genera previously referred to are wholly in accord with this view. In this connection it is interesting to note how those workers, attack-

ing the problem in *Crepis* from an experimental standpoint, arrived at the same conclusion in regard to the cause of polyploidy. A geneticist's (BABCOCK 3) view is as follows:

point mutations and chromosome aberrations seem to be so very rare that they could hardly suffice for an explanation of the origin of the thousands of species, genera and families of organisms. Point mutations may start polymorphism, but hybridization is necessary to produce polyploidy.

Again, NAWASCHIN (40), after making a detailed study of the individual ground sets of chromosomes in the polyploid series of *Crepis*, concludes that hybridization is the only explanation of the various assortments in different species.

TETRAPLOIDY

In deriving a polyploid series of individuals by the crossing of those with different multiples of chromosomes in their nuclei, the origin of the first multiplication of the normal diploid complement, that is, the tetraploid, is naturally of vital importance. The widespread appearance of tetraploids is everywhere evident. Table I shows the large number of species in this category. DENHAM (12) finds that the Asiatic cottons are normal diploids, but that the New World and Egyptian cottons are tetraploid. Our most common species of blueberries are tetraploid (LONGLEY 36). Many other instances could be cited, such as those in *Rosa* and *Rubus*, to which reference has already been made.

In most experimental cases, tetraploidy is known to be a result of hybridization. The famous "gigas" forms of *Oenothera lamarkiana* are clearly hybrids, as shown by their chromosome behavior, and are tetraploids (DAVIS 11). More recently, HÅKANSSON (19) has shown that in hybrids of the cross *O. lamarkiana* \times *O. biennis*, both haploid and diploid pollen mother cells may occur in the same anther sac. The case of *Primula kewensis* and that of the tetraploid species of *Datura* are other well known examples of a doubling of diploid complement arising as a result of crossing. The most recent and convincing work in this connection is that of KARPECHENKO (29). In a cross between species of *Raphanus* and *Brassica*, the F_1 generation gives rise to some plants that produce gametes with the somatic complex of chromosomes (doubling having taken place from a failure

of the mates to pair and initiate the reduction division). The F_2 generations of such types are tetraploid and do not revert. KARPECHENKO states:

Having like the F_1 hybrids a pod of very peculiar structure, which characterizes them as distinct species, the tetraploid F_2 hybrids acquire quite regular reduction divisions, full fertility, and moreover, prove unable to cross with one of their parents—*Brassica*. It seems that we here approach nearer than we ever did the experimental reproduction of one of the processes in species formation.

In connection with the consideration of the doubling of a given chromosome complement, there has been much speculation to show that the doubled number represents merely a duplication of the fundamental set. Of course, such a chromosome doubling takes place in cases of apogamous reproduction, but there is no evidence to show how tetraploid or polyploid individuals arise in consequence. SAX (45) does not see how differential chromosome matings involving unpaired members or univalents can occur in *Triticum* hybrids if polyploidy is the result of a mere duplication of the original set.

The theory of the origin of tetraploidy that thus far seems most plausible in the light of available evidence is that of the "indirect chromosome union" put forward by WINGE (53). It is conceivable that in a given cross there will be no pairing of chromosomes derived from the parent gametes at the time of the reduction division. Yet, if the chromosomes are to find a partner, then each of the chromosomes in the zygote must divide, for thus indirectly to produce a union of chromosomes and we must assume that this is realized in the hybrid zygotes that have any possibility at all of propagating. The hybrid sporophyte thus produced will then have $4x$ chromosomes.

WINGE's theory will be seen to differ from other theories of chromosome doubling in that it brings into play the act of hybridization, the resultant chromosome incompatibility producing the duplication of haploid sets which on segregation give rise to a new qualitative as well as quantitative assortment of chromosomes in the offspring. This theory finds strong support in the work of KARPECHENKO just mentioned.

LAGGING UNIVALENTS

Having considered quantitative differences in the chromosome equipments, namely, polyploidy as a criterion of hybridization, the

equally important qualitative differences as ear marks of the same situation may now be discussed. The presence of unpaired or univalent chromosomes is one of the most striking indications of a hybrid. Such chromosomes are well instanced in the case of *Spartina alterniflora* var. *glabra*.

The classic example of lagging univalents is ROSENBERG's (43) *Drosera* hybrid, in which ten univalents lag on the spindle and follow a random distribution to the poles in both divisions. Some univalents may be extruded, resulting in polycary and polyspory. Experimentally produced hybrids often show similar chromosome behavior, particularly in triploids resulting from crosses of tetraploids with diploids. Among these may be cited *Papaver somniferum* \times *P. orientale* (YASUI 54), and *Nicotiana sylvestris* \times *N. tabacum* var. *purpurea* (GOODSPEED and CLAUSEN 18), the first species being the diploid in each instance. A very striking case in nature is seen in a hybrid *Isoetes* (JEFFREY and HICKS 28). Analogous cases also occur naturally in *Senecio* (AFZELIUS 2) and *Hieracium* (ROSENBERG 44).

In *Spartina*, as well as *Papaver* and *Hieracium*, some of the univalents appear to split in the heterotypic division. In *Viola* crosses CLAUSEN (6) reports conditions from a splitting of part of the univalents to instances where they all divide in the first division after the bivalents have passed to the poles. This latter situation is typical in triploid *Triticum* hybrids (THOMPSON 49).

NON-PAIRING

Several species of grasses in this research present another category of univalent chromosomes in which the numerical complement of the nucleus furnished each chromosome with a mate, but a few do not pair because of some lessened affinity existing between them. It has been noted in *Alopecurus pratensis*, for example, that one or two sets of chromosomes are seen unpaired in the diakinesis, and, further, that these may lag on the heterotypic spindle in this condition, at times being extruded into the cytoplasm. *Dactylis glomerata* presents a similar situation, in which the late diakinesis may be found with all the chromosomes unpaired, not a few of these lagging as univalents on the spindle. *Phalaris arundinacea* var. *picta* consistently displays unpaired mates as laggards and extrusions.

Phenomena analogous with the foregoing have been reported in certain varietal forms of *Oryza sativa* (KUWADA 30). DAVIS (11) noticed an unpaired chromosome set consistently appearing in the diakinesis of *Oenothera biennis*. The same condition has been shown since to be common in several other species of this genus, famous for its hybrid members (CLELAND 7). Some of the diploid species of *Rosa* whose ancestry might be questioned as to purity also display unpaired chromosomes in the early stages of the maturation divisions.

LAGGING BIVALENTS

A lack of uniformity in the movements of chromosomes during the maturation division is not dependent on the presence of univalents, however. Many of the species examined in this study showed bivalent laggards. The three species of *Festuca*, and *Avena sativa*, *Ammophila breviligulata*, and *Alopecurus pratensis*, all display lagging bivalents at both metaphase and anaphase of the heterotypic division. Lagging and extrusions paralleling these instances may be seen in many of the cases cited in the discussion of polyploidy, such as *Rosa*, *Rubus*, *Crataegus*, *Fragaria*, *Primula*, and *Gossypium*. The same meiotic aberrations have recently been correlated with polycary and polyspory in the pentaploid and hexaploid species of *Vaccinium* (LONGLEY 36) and in the several species and varieties of *Aesculus* (HOAR 22).

UNEQUAL DISTRIBUTION

Extreme conditions of lagging bivalents often may be operative in effecting an unequal segregation of chromosomes to the poles in the heterotypic anaphase. More specifically, one or more lagging bivalents remain at or near one of the poles during the metaphase, and if not extruded, will be included in the subsequently formed nuclei. Striking instances of this phenomenon have been observed in *Alopecurus geniculatus* var. *aristulatus*, *Avena sativa*, and *Phalaris arundinacea* var. *picta*. Conditions similar to these are instanced in several varietal forms of *Citrus* (FROST 16), and in *Potentilla anserina* var. *grandis* (ROSCOE 42), where the situation is complicated still further by the presence of univalents.

POLLEN VIABILITY

The common result of extruded chromatin from irregular divisions in hybrids such as have been cited is the appearance of polycary and polyspory and consequently sterile pollen. It is noteworthy that in the grasses investigated polyspory has not been observed, yet polycary and sterile pollen are frequent in appearance. A striking parallel situation is seen in wheat hybrids, where polyspory is absent in the presence of polycary, and 98-100 per cent of the pollen is sterile (THOMPSON 49).

The case of *Avena sativa* displays the unusual condition of apparently perfect pollen in the presence of a considerable amount of polycary. The so-called "fatuoid mutants" of this obviously hybrid species show the same degree of viable pollen production (HUSKINS 24). Of course known hybrids often have perfectly good pollen, yet a considerable amount of work on wheat hybrids (THOMPSON 49) has demonstrated that a high percentage of the apparently good pollen will not germinate, and, further, that in the case of seeds derived by the use of such pollen as will germinate, 50 per cent of the offspring never mature.

CYTOMYXIS

In a discussion of the nuclear phenomena of plant hybrids, we have thus far noted and considered polyploidy and irregularities in chromosome behavior with the resultant degenerate condition of the germ cells. There is still another apparent abnormality which has been observed extensively in the pollen mother cells of the grasses in this study, and that is cytomyxis. More convincing evidence as to the occurrence of cytomyxis in connection with hybrids is offered by an investigation of the grasses in the tribe Paniceae, and for this reason the discussion of the phenomenon will be left for a later article.

Conclusions

FESTUCEAE

Festuca.—The relatively high degree of polyploidy in the species studied (*F. ovina* twenty-eight, and *F. rubra* and *F. duriuscula* both twenty-one) is quite consistent with the size of the genus. There are about one hundred species known, forty of which are in the United

States. The varieties of *F. ovina* (TURESSON 51) and *F. rubra* are legion, and they are reported to cross with one another freely (COCKAYNE in LOTS 37). Taxonomic characters designed to distinguish these two common species are not at all clearly demonstrable in many instances. The presence of lagging chromosomes, polycary, and sterile pollen witnesses the hybrid origin of all three of the particular strains of the species investigated.

Dactylis glomerata.—Hybrid criteria are not outstanding in this species, yet tetraploidy, sterile pollen, lessened chromosome affinity, and the presence of two other species in the genus (including many European varieties, HEGI 20) taken as a whole make the hybrid origin of this species seem quite probable.

AVENEAE

Avena sativa.—This is a rather large genus of about fifty-five known species. The hybrid origin of many cultivated forms of this species has already been indicated by the work of HUSKINS (24). This escaped variety shows lagging and polycary to an even greater degree than in those heretofore reported.

AGROSTIDEAE

Ammophila breviligulata.—As in *Dactylis*, this is a small genus but with a somewhat wide dispersal, the two European species being distinct from the North American ones. Lagging chromosomes and a large amount of sterile pollen as well as tetraploidy bear witness to a hybrid origin of this species, however remote its occurrence.

Alopecurus.—Both *A. pratensis* and *A. geniculatus* may run to varieties (HEGI 20) in this genus of twenty-five species, eight of which are found in the United States. Cytological evidence of hybridity, mostly in the form of lagging chromosomes and sterile pollen, is abundant in the diploid variety of *A. geniculatus*, and only less marked in the tetraploid *A. pratensis*.

CHLORIDEAE

Spartina.—This is a rather extensive genus of fourteen species, mostly in North America. The hexaploid *S. alterniflora* var. *glabra* with its fourteen univalent chromosomes is undoubtedly a hybrid.

Cytological evidence of crossing is rather meager in *S. michauxiana*; still an appreciable amount of bad pollen together with tetraploidy suggests hybridity.

PHALARIDEAE

Phalaris.—There are two distinct taxonomic sections in this genus of about twenty species in Europe and the United States. *P. canariensis*, of the section Euphalaris, does not bear marked evidence of a hybrid origin, other than a small percentage of sterile pollen and a possible aberration from the normal seven basis in the haploid count of six. In the section Digraphis, *P. arundinacea* is undoubtedly a pure, diploid species. This species may run to some five varieties in Europe (Hegi 20), and the cultivated tetraploid variety *picta* clearly shows its hybrid origin by the presence of lagging chromosomes and an appreciable amount of sterile pollen.

Summary

1. The following members of the tribes Festuceae, Aveneae, Agrostideae, Chlorideae, and Phalarideae have been investigated.

Diploids: *Alopecurus geniculatus* var. *aristulatus*, *Phalaris canariensis* *P. arundinacea*.

Tetraploids: *Dactylis glomerata*, *Ammophila breviligulata*, *Alopecurus pratensis*, *Spartina michauxiana*, *Phalaris arundinacea* var. *picta*.

Hexaploids: *Festuca rubra*, *F. duriuscula*, *Avena sativa* var. *mutica*, *Spartina alterniflora* var. *glabra*.

Octoploid: *Festuca ovina*.

2. Lagging univalents are found in *Spartina alterniflora* var. *glabra*.

3. *Phalaris arundinacea* is considered a pure, diploid species.

4. Varying degrees and combinations of non-pairing, lagging and extrusion, caryomyxis, polycary, and sterile pollen are found in all other species.

5. No cases of polyspory are noted.

6. Polyploidy or cytological abnormalities of the maturation divisions or both are considered as evidence of the hybrid origin of all species except *Phalaris arundinacea*.

This research has been carried out at the suggestion of Professor E. C. JEFFREY, to whom I am greatly indebted for advice in its prosecution.

LABORATORIES OF PLANT MORPHOLOGY
HARVARD UNIVERSITY

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EXPLANATION OF PLATES XXV-XXVII

PLATE XXV

× 2300 diameters

Festuca duriuscula L.

FIG. 1.—Heterotypic metaphase showing lagging and extruded bivalent.

FIG. 2.—Heterotypic anaphase with lagging univalents and extruded bivalents.

FIG. 3.—Interkinesis; diad split nearly completed; chromatin connections between nuclei of adjacent mother cells.

FIG. 4.—Diad stage practically completed; chromatin in path of obliterated spindle and excluded from the nuclei.

FIG. 5.—Homeotypic divisions; no irregularities.

Festuca ovina L.

FIG. 6.—Heterotypic metaphase; laggards and extrusions.

FIG. 7.—Late heterotypic anaphase; lagging bivalents at equator.

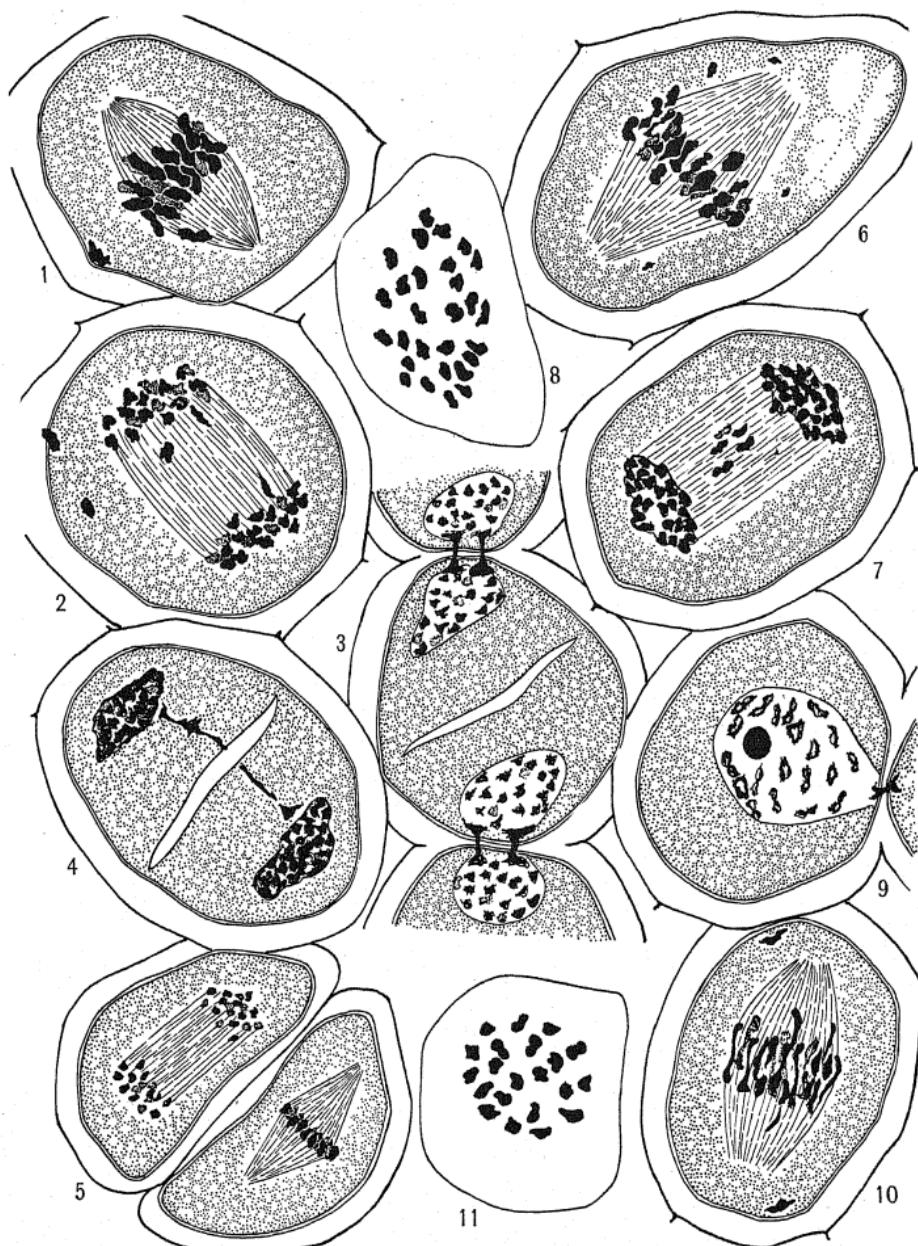
FIG. 8.—Heterotypic metaphase, polar view, showing twenty-eight bivalents at plate.

Festuca rubra L.

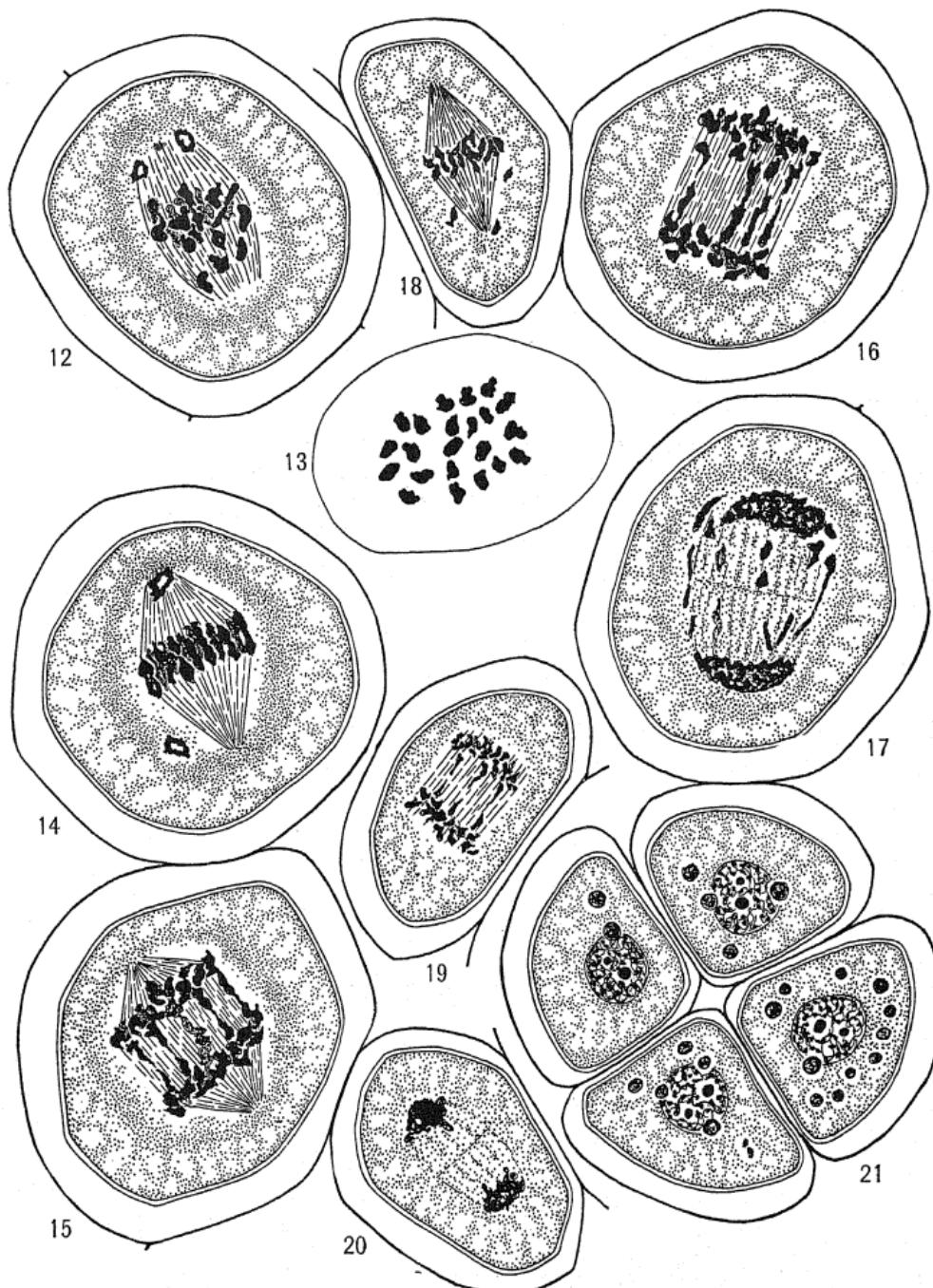
FIG. 9.—Diakinesis, twenty-one pairs, one partly stranded in adjacent mother cell.

FIG. 10.—Heterotypic metaphase with extruded chromosomes.

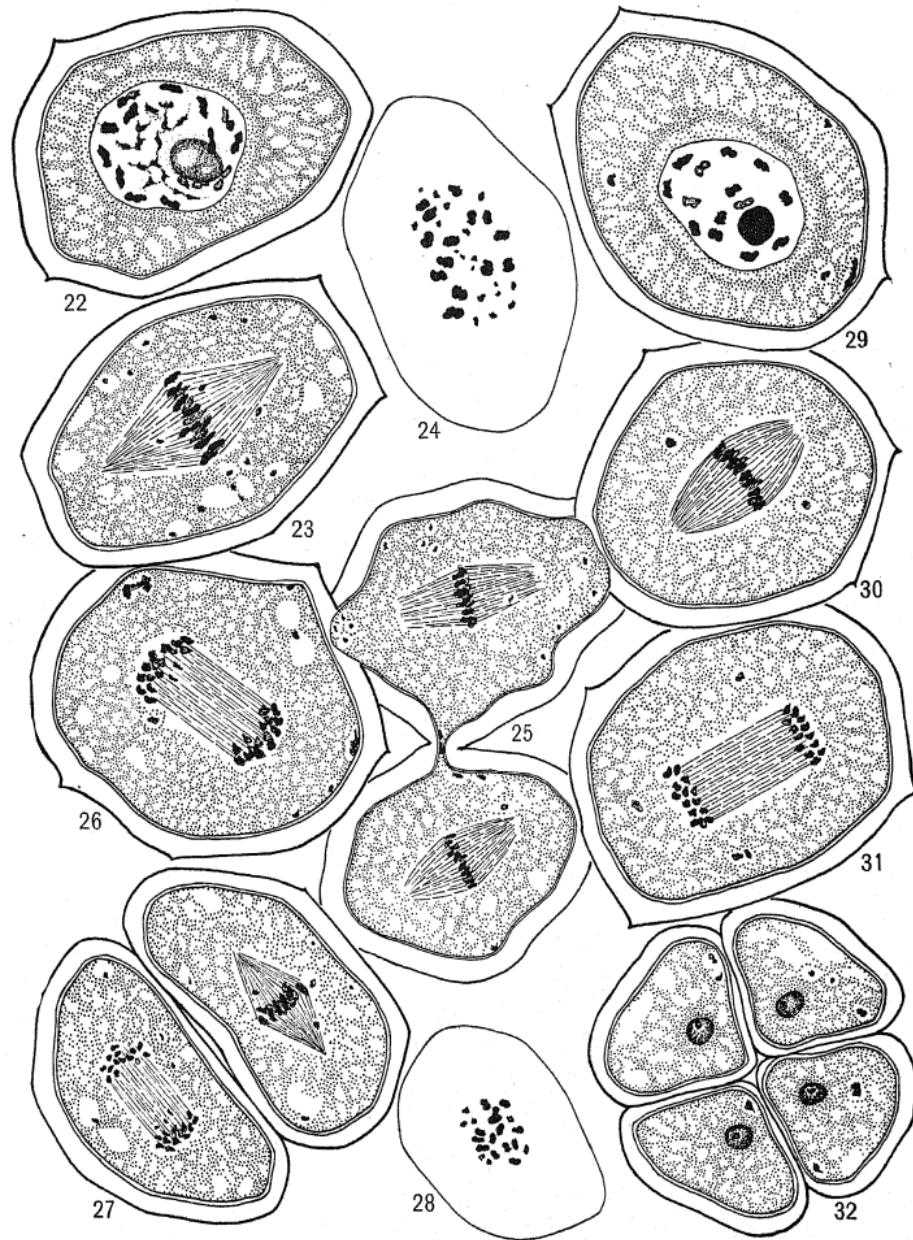
FIG. 11.—Heterotypic metaphase, polar view, showing twenty-one bivalents.











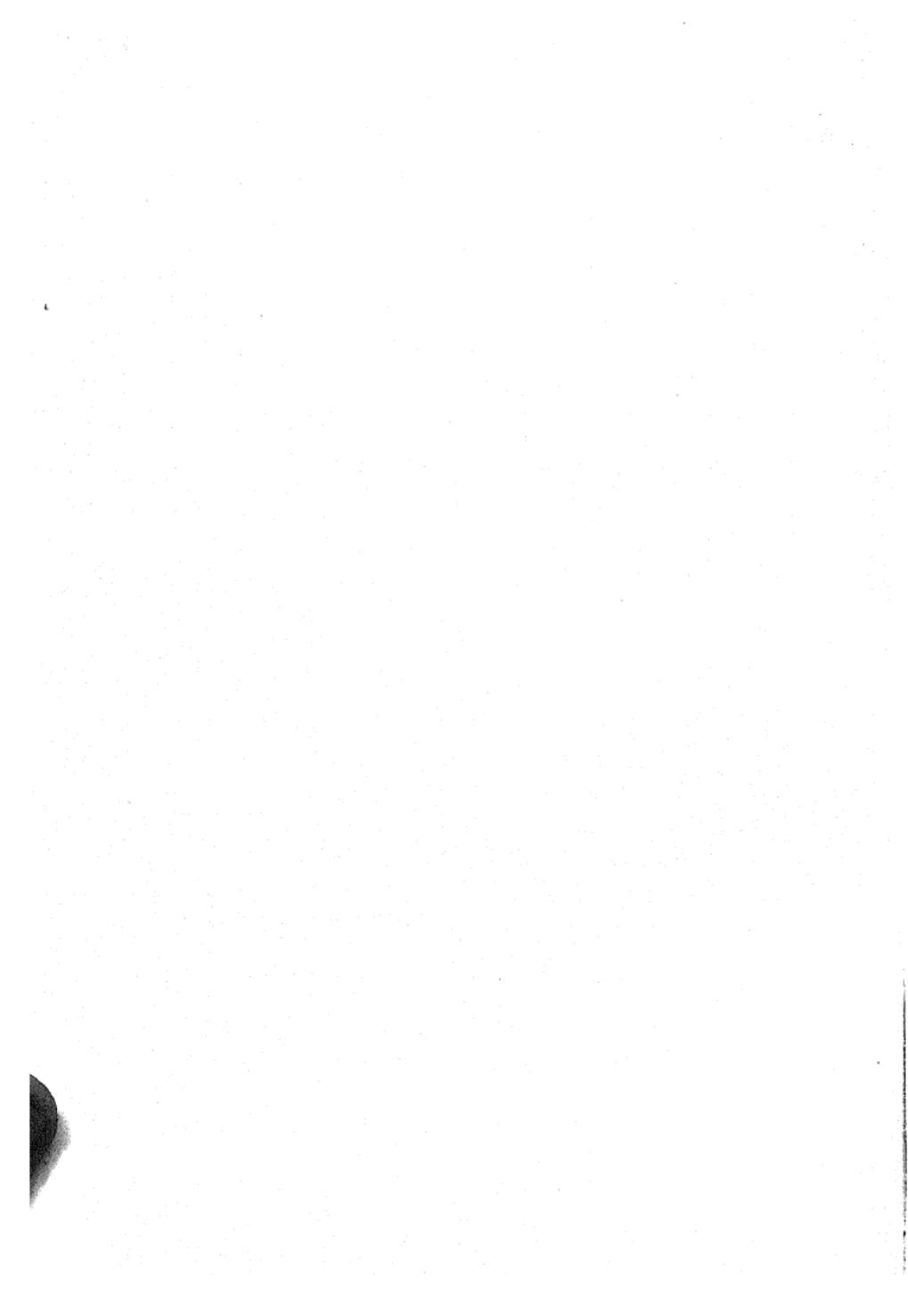


PLATE XXVI

 $\times 1900$ diameters*Avena sativa* L.

FIG. 12.—Early heterotypic metaphase; laggards, especially ringed bivalents at poles.

FIG. 13.—Heterotypic metaphase; polar view showing twenty-one bivalents.

FIG. 14.—Late heterotypic metaphase; note lagging and extruded bivalent.

FIG. 15.—Early heterotypic metaphase; five pairs of chromosomes late in splitting.

FIG. 16.—Late heterotypic anaphase still showing laggards.

FIG. 17.—Heterotypic telophase; laggards excluded from nuclei.

FIG. 18.—Homeotypic metaphase; note extrusions.

FIG. 19.—Homeotypic anaphase showing laggards.

FIG. 20.—Homeotypic telophase; some chromosomes barely reached nuclei.

FIG. 21.—Tetrad showing considerable polycary.

PLATE XXVII

 $\times 2300$ diameters*Spartina alterniflora* Loisel var. *glabra* (Muhl.) Fern.

FIG. 22.—Diakinesis showing fourteen bivalents and fourteen univalents in center of nucleus.

FIG. 23.—Heterotypic metaphase; note many extruded univalents.

FIG. 24.—Heterotypic metaphase, polar view, showing fourteen bivalents, eleven univalents, and three split univalents.

FIG. 25.—Two mother cells showing cytomyxis $\times 1540$.

FIG. 26.—Heterotypic anaphase; note coalescing and extruded univalents.

FIG. 27.—Homeotypic divisions; univalents still presenting irregularities.

FIG. 28.—Homeotypic metaphase, polar view, showing univalents and bivalents at plate.

Spartina michauxiana Hitch.

FIG. 29.—Diakinesis, showing fourteen bivalents; note chromatin in cytoplasm.

FIG. 30.—Heterotypic metaphase, presenting regularity except for persistent cytoplasmic chromatin.

FIG. 31.—Heterotypic anaphase; again regularity except extrusions.

FIG. 32.—Tetrad showing polycary and vacuolated condition of constituents.

MOTTLE-LEAF IN CITRUS ARTIFICIALLY PRODUCED BY LITHIUM¹

A. R. C. HAAS

(WITH FOUR FIGURES)

The mottle-leaf problem is an intricate one not readily understood, owing to the fact that artificially in controlled cultures it has been difficult to produce the mottling satisfactorily over a period of time by means of chemically pure salts, and to the fact that it has been found difficult to improve trees badly affected with mottle-leaf in the field. KELLEY and CUMMINS (6) have found the composition of mottled citrus leaves from the field to contain larger amounts of potassium, phosphorus, and frequently of nitrogen, but smaller amounts of calcium than normal leaves. The composition of mottled citrus leaves resembles that of immature citrus leaves.

The writer has attacked the mottle-leaf problem in three ways: (1) Poisoning the leaves so as to keep them from becoming mature from the standpoint of composition; (2) replacement of the calcium of the soil with different bases and leaching out of much of the liberated calcium; (3) locking up much of the supply of soluble calcium in the soil and possibly within the plant by the use of alkaline substances without leaching out the calcium from the soil.

In a recent paper, HAAS, BATCHELOR, and THOMAS (3) have pointed out in connection with yellows or little-leaf of walnut trees that the absorption of a toxic agent in sufficient concentration may be an important factor in producing such diseases. In the present paper the writer will report experiments dealing with the first method of approach, and will deal with the effects of lithium as a toxic agent in the artificial production of mottle-leaf of citrus.

So far as the writer is aware, no report has been made of lithium as a constituent of mottled citrus leaves in the field. Whether or not upon investigation of mottled leaves in the field lithium is found to be present, the data nevertheless are of scientific value in elucidating the nature of the physiological disease called mottle-leaf.

¹ Paper no. 194, University of California, Graduate School of Tropical Agriculture and Citrus Experiment Station, Riverside, California.

Lithium has the lowest atomic weight of the non-gaseous elements in Group I in the periodic system, and has long been used in physiological laboratories in studies involving ascent of sap, etc., because of its ease of movement and qualitative determination. The writer has also concluded experiments in which other elements of low atomic weight have been employed, and has succeeded in artificially producing mottle-leaf of walnut trees in the field, but these results must be presented at a later time.

When a concentration of lithium equal to 0.2 parts per million was used in conjunction with similar amounts of B, Al, I, Ti, Br, Sr, Mn, and NH_4 (HAAS and REED 4) in the culture solution for the growth of citrus in sand, the trees grew in a normal manner. In order to obtain evidence in regard to the rôle of each constituent of this "A-Z" solution that was used in overcoming the inorganic deficiencies of Hoagland's solution (REED and HAAS 7) for the growth of citrus, sand cultures were conducted in galvanized iron containers 18 inches diameter \times 26 inches deep. Orange and lemon trees budded on sour-orange stock were planted in these containers, and a single but different constituent was omitted from the "A-Z" solution in each series. The strength of the modified "A-Z" solutions that were used in Hoagland's solution in these cultures was five times that of the "A-Z" solution originally used. The first application of these culture solutions was made at the time of planting the trees, so that the new roots were subject to the treatment from the very beginning of growth. Comparison was possible with many other sand cultures of citrus growing at the same time.

One Valencia orange and two Eureka lemon trees comprised each series. After one year's growth, the leaves of the lemon trees, in all but one series, began to wilt and absciss, beginning with the oldest leaves; while the orange trees maintained excellent growth. Fig. 1 shows the vigorous growth of the orange trees (left) in each series, and the permanently wilted condition of the leaves of the lemon trees (center and right) in each series except one. The only series in which the lemon trees made good growth was that in which lithium had been omitted ("A-Y") from the "A-Z" solution. The wilted condition of the leaves of the lemon trees that had received a culture solution containing 1 p.p.m. of lithium was very striking,

and was not related to the supply of moisture in the sand. Upon removal of the trees from the sand, it was found that the roots had become soft and gelatinous. The results suggested that the lithium was toxic and that Valencia orange trees were less sensitive than Eureka lemon trees to this toxic agent. The toxicity of this small concentration of lithium was the greater owing to the fact that the

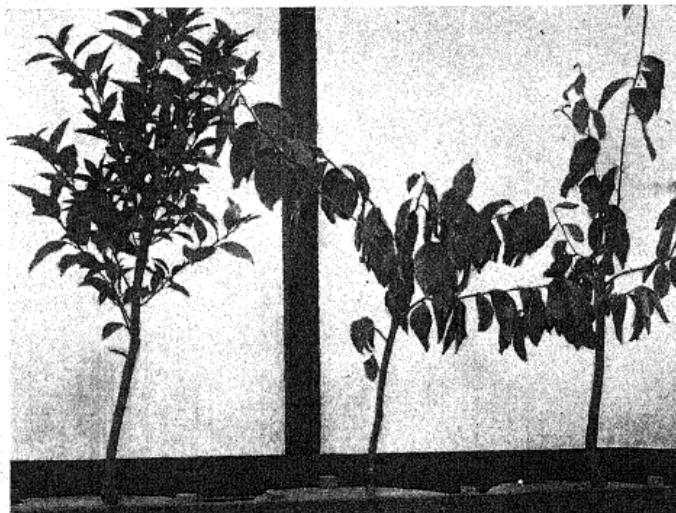


FIG. 1.—Left, Valencia orange tree typical of sand cultures receiving Hoagland's culture solution containing 1 p.p.m. of certain elements not ordinarily added to culture solutions (five times "A-Z" concentration) or lacking any one of elements of "A-Z" solution; center and right, Eureka lemon trees permanently wilted in sand culture when culture solution contained 1 p.p.m. of lithium regardless of other elements of "A-Z."

culture solution containing lithium was applied from the beginning of growth, when the pruned tree consisted of only the lower two-thirds of the bare trunk and a clean main root about a foot in length.

In another experiment with citrus tree cultures in containers such as were previously described, budded Eureka lemon trees were given Hoagland's culture solution and were allowed to grow for several months, after which 25 p.p.m. of lithium was added to the culture solution. Many of the new leaves began to mottle as they became older, and showed dried areas along the leaf margins as

well as in the mottled portion, as shown in fig. 2. As a rule, leaves that were already mature did not mottle but became dry along the leaf margin and between the veins. As the drying of the leaves became severe, many of them abscised. The new leaves were much wrinkled, and were pale yellowish green except near the midrib, where they were of a darker green (fig. 3). Upon removal of the lithium-containing culture solution from the sand and its replacement with a similar solution lacking lithium, new growth developed

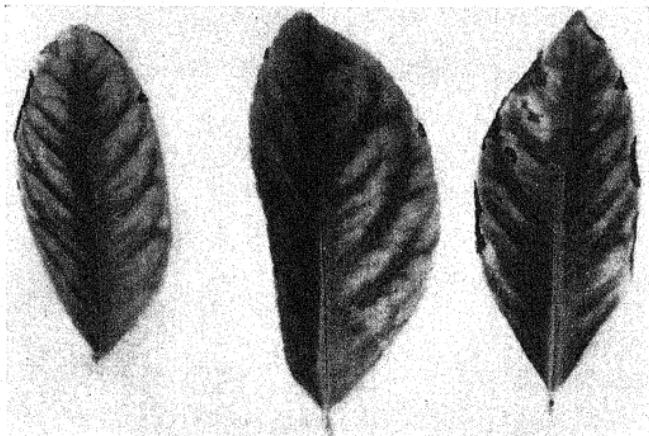


FIG. 2.—Toxic effects of lithium on lemon trees in sand culture

and the leaves of the successive cycles of growth were less and less wrinkled, until finally normal growth was produced.

No effects of lithium upon the external appearance of the citrus roots have been observed, and upon removal of the lithium from the solution bathing the roots, the root growth is resumed provided other environmental conditions are favorable. It appears that, with a toxic agent such as lithium, which is not readily precipitated within the conducting system of the tree, after equilibrium is established within the tree the toxic agent concentrates in the leaves as a result of transpiration. Consequently upon removal of the toxic agent from the solution bathing the roots, the dilution of the toxic agent

takes place most rapidly in the roots, with the result that the root growth then made is most important to the recovery of the tree. The plant evidently gets rid of the lithium contained within its conducting tissues largely through the production of new growth, rather than by concentrating all of the toxic agent in the already injured leaves.

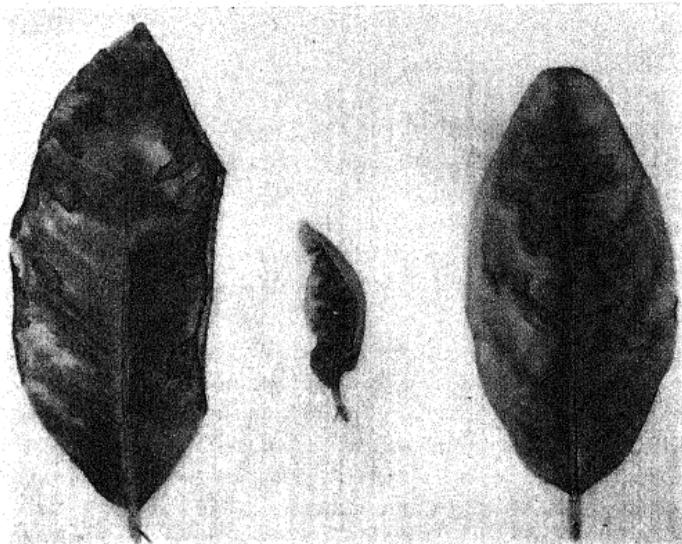


FIG. 3.—Toxic effects of lithium on lemon leaves in sand cultures; left, mature leaf burned between veins and along margin; center and right, chlorotic, crinkly, and thin new leaves.

The assumption is made that the lithium accumulations between the veins and along the margins of the veins are unequal in amount, and affect growth to different degrees as a result of such unequal depositions. As a consequence the leaves become badly crinkled, regardless of their size. FAWCETT and LEE (2) have described a crinkly leaf condition of lemon trees in California, and point out the possible relationship between this condition and insect effects, as well as the possible hereditary transmission of such abnormalities. The crinkly leaf condition seen in fig. 3 has been produced by a

known cause, and the results add to our knowledge of the causation of observed physiological effects. Additional experiments have also shown that a crinkly leaf condition of lemon leaves may be produced by large concentrations of nitrate in the solution bathing the roots. In this case it is reasonable to assume rapid but unequal rates of growth of tissues, with different capacities to utilize the abundant nitrate.

The effect of lithium on citrus trees was then studied in soil cultures in 10 gal. earthenware jars, the soil used being that classified as Sierra loam, which was obtained near the Citrus Experiment Station. The leaves and shoots of the budded Valencia orange trees were pruned off, but the washed root system was left intact when the trees were planted in the jars. The cultures were grown in the glasshouse from April until the following March. Immediately after the trees were planted in the jars, the soil in each jar received 2 liters of Hoagland's culture solution containing respectively 0, 3, 6, 9, 12, and 15 parts of lithium per million parts of dry soil, followed by 2 liters of a similar solution in which lithium was absent. Although the culture jars had drainage outlets, no drainage water was allowed to escape. In case of drainage, the solution was returned to the jar from which it came. The experiment was begun in April, and by the following February all of the trees had died except the control trees and the one in soil containing 3 p.p.m. of lithium. The control trees were normal in appearance, while the tree in the culture containing 3 p.p.m. of lithium had many burned leaves which fell prematurely. Some leaves were small, and their appearance resembled that of mottled leaves. Every leaf of the trees in cultures that received 6 p.p.m. or more of lithium, burned and was shed prematurely. The succeeding leaves fared no better than their predecessors. Finally all of the shoots died back to the trunk.

The toxicity of lithium was severe, even though only one application of solution containing lithium was made during the 11-month experimental period. Samples of burned leaves were obtained by shaking the tree trunks and catching the falling leaves before they touched the soil. Analyses of these leaves by Mr. S. M. BROWN, Assistant Chemist in the Agricultural Chemistry Division of this Station, showed that the amount of lithium in the burned leaves was

too small to be detected gravimetrically, but that spectrum analysis gave positive tests for its presence in the affected leaves.

Water cultures in quart Mason jars were begun on April 19 with five sweet orange seedlings per jar and three jars in each series. Hoagland's nutrient solution was used, and lithium additions were made so as to have concentrations from 0 to 7 p.p.m. of lithium in the culture solution. On the following June 1 there was slight marginal discoloration and burning in the 2 and 3 p.p.m. concentrations, with severe burning at the higher concentrations. Similar water cultures were conducted with lemon seedlings from April 27 until the following June 20. At 1 p.p.m. there was a slight burning at the tip and along the leaf margin, and the new leaves were very chlorotic. At higher concentrations these effects were more severe, with abscission of most of the leaves and ultimate death of the seedlings.

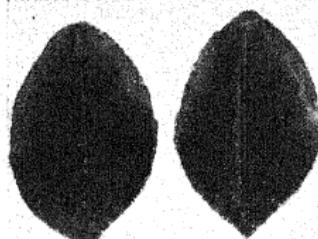


FIG. 4.—Toxic effect of lithium on leaves of lemon seedlings; leaf tip and margin become dull green and burn very rapidly after discoloration; abscission of burned leaves most rapid.

A field experiment was begun in October, 1926, at the Rubidoux tract of the Citrus Experiment Station, in which lithium nitrate was applied to the trees in basins. A row of Smith's Early navel orange

trees on sweet-orange rootstock (C. E. S. no. 46) were selected as being uniformly good trees, relatively free from mottle-leaf throughout the entire row. The trees were approximately 20 years old. The upper three trees in the row were irrigated by means of furrows, and the remainder were irrigated by the quartered basin method. The first or uppermost basined tree, together with the three furrow-irrigated trees, served as controls. The other basined trees, beginning with the tree below the controls, received respectively lithium nitrate ($\text{LiNO}_3 \cdot 3\text{H}_2\text{O}$) in the following amounts: 25, 50, 75, 100, 150, and 200 gm. Applications of these amounts were made on the following dates: 10/15/26, 2/2/27, 6/20/27, 7/23/27, 8/30/27, 10/21/27, 3/12/28, and 5/1/28, eight applications in all. In a single applica-

tion the lithium applied was 1.41, 2.82, 4.23, 5.64, 8.46, and 11.28 gm. respectively. Eight applications, therefore, have given the basins 11.28, 22.56, 33.84, 45.12, 67.68, and 90.24 gm. of actual lithium respectively during the course of the experiment. The trees have gradually been losing many of their leaves at the higher concentrations, and the remaining leaves are badly mottled. At the lowest concentration of lithium there is only a small amount of mottle-leaf, the tree being not appreciably affected as yet. At the second lowest concentration many of the leaves are curled from the apical end toward the ventral side. Many of the leaves are undersized, willow-like in outline, and severely mottled. Above this concentration the greatest apparent change is in the increasing loss of leaves. Some of the leaves on the trees that received the larger concentrations were burned near the tip prior to abscission.

In order to get a better understanding of the nutrition of normal and mottled leaves, studies were made on sap obtained by means of air suction upon comparable portions of normal and mottled citrus trees. The method employed was essentially that described by BENNETT, ANDERSSEN and MILAD (1), the sap being withdrawn by means of a battery of suction flasks as the plant material was cut shorter and shorter. The material consisted of the trunks of twelve normal Valencia orange trees 2 to 3 years of age, and of two sets of twelve each of badly mottled Valencia orange trees also 2 to 3 years of age; all trees having been budded on sour-orange stocks. Sap was also withdrawn from the twelve roots of the normal trees and from the lot of twenty-four roots of the mottled trees. Twenty-four branches, $1\frac{1}{4}$ – $1\frac{1}{2}$ inches in diameter at the larger end, were taken from normal and an equal number from badly mottled Washington navel orange trees budded on sweet-orange stocks, at the Rubidoux tract of the Citrus Experiment Station, and their sap extracted by the method described. The sap obtained in each case was ashed and a partial analysis was made for sodium, potassium, calcium, and magnesium. The results obtained are given in table I, where it is seen that the sap of the trunks of mottled Valencia orange trees and of the branches of mottled Washington navel orange trees was not lower in calcium content than the sap of comparable portions of normal

trees, although a smaller amount of calcium was found in the sap of the roots of the mottled than in that of the roots of the normal trees. Sap analyses are as yet difficult of interpretation, because we do not know how rapidly the bases found in the tracheal sap are renewed by root absorption when they are removed from the sap by the leaves or other plant organs.

The absorbed lithium is assumed to act as a poison to the leaves, preventing them from changing their ash composition from that characterizing immature leaves to that of mature leaves. In this

TABLE I

COMPOSITION OF SAP OBTAINED BY SUCTION FROM COMPARABLE PORTIONS OF
NORMAL AND MOTTLED CITRUS TREES (PARTS PER MILLION)

| | TRUNKS OF VALENCIA ORANGE TREES, 2-3 YEARS OLD, BOX SPRINGS TRACT, CITRUS EXP. STA., MARCH 24, 1927 | | | ROOTS OF VALENCIA ORANGE TREES, 2-3 YEARS OLD, BOX SPRINGS TRACT, CITRUS EXP. STA., MARCH 24, 1927 | | | BRANCHES (1 $\frac{1}{2}$ -1 $\frac{1}{2}$ INCHES DIAM.) OF 21-YEAR-OLD NAVEL ORANGE TREES, RUBIDOUX TRACT, CITRUS EXP. STA., APRIL 7, 1927 | |
|----------|---|---------------|---------------|--|---------------|--------|---|--|
| | Normal | Badly mottled | Badly mottled | Normal | Badly mottled | Normal | Badly mottled | |
| Ash..... | 546 | 347 | 449 | 914 | 738 | 452 | 665 | |
| Na..... | 287 | 252 | 303 | | 482 | 66 | 52 | |
| K..... | 230 | 145 | 174 | | 253 | 91 | 151 | |
| Ca..... | 81 | 105 | 119 | 201 | 155 | 80 | 126 | |
| Mg..... | 38 | 35 | 40 | 52 | 68 | | | |
| pH..... | | | | | | 6.2 | 6.0 | |

case it is assumed that the toxic substance prevents the leaves from obtaining the requisite amount of calcium, etc., for the normal functioning of chlorophyll. If this is true, then the affected leaves should be unable to utilize the available calcium, etc., even though an abundance be at hand, somewhat as in the case of cyanide poisoning in which the individual breathes heavily to get an abundance of oxygen but the system cannot utilize it and death occurs from asphyxiation. We may consider two possibilities as the modus operandi. (1) The toxin may be supposed to act directly on the chlorophyll to break it down and bring about the mottling, the inorganic constitution being only that occurring in the leaves at the time the mottling develops. In this case mature leaves having the full complement of calcium, etc., should be found to mottle, which is not the case. If they show injury at all they do not mottle but burn and

absciss. This fact might be explained on the supposition that the mottling stage has been passed by so rapidly as to obscure its appearance, but this hypothesis is not tenable on account of the fact that in many such cases the burning is at the tip rather than between the veins. Burning between the veins is often preceded by mottling, although the mottling may be of short duration. Mature leaves are not known to mottle if normal up to the time of becoming mature with respect to their inorganic composition. (2) It may be assumed that it is the inorganic constitution of the leaves brought about by the toxin that determines whether or not the leaves mottle. This hypothesis is supported by the fact that in unpublished data on the growth of citrus cuttings in water cultures and in other types of cultures, mottling has been brought about by certain modifications of the inorganic nutrition of the plants. Furthermore, when desiccating winds defoliate normal orange trees, frequently some of the succeeding growth may be considerably mottled. This has been attributed by HAAS and REED (5) to losses of inorganic constituents from the portions of the trees that normally furnish new leaves with requisite amounts at the proper rate. The second view does not explain why some normal appearing leaves occur on badly mottled trees and yet have the composition of immature or mottled leaves. This fact was long a serious obstacle in the interpretation of analytical data from affected material for which the normal or control portions were obtained from the affected trees rather than from entirely normal trees. Possibly the chlorophyll in some leaves may be able to resist a wider range in inorganic constitution than that of other leaves on the same tree, or perhaps these normal appearing leaves are soon to mottle.

The former view assumes a direct effect of the toxin on the chlorophyll molecule; the other view supposes an indirect action upon the processes of chlorophyll formation and decomposition through the inorganic constitution of the protoplasm of the leaf tissues.

It is not to be concluded that all leaf poisons will produce the same type of effect upon the chlorophyll of the leaves. It is definitely known from unpublished results that the effects of different poisons may be slightly different from one another, and that there are various types of mottle-leaf. In each case the inorganic composi-

tion of the mottled leaves is typical of that found in immature normal leaves.

Different toxic substances may move to different locations in the leaf: some toxins may affect the leaf near the apical region, and if in sufficient concentration may also affect the basal region; whereas, other toxins may act between the veins over the entire leaf area from the beginning of visible injury.

There is no a priori reason why certain organic substances may not be found that may affect citrus leaves in the manner that lithium does. This seems probable in view of the fact that frequently on certain soils continued use of certain organic fertilizers has been accompanied by severe cases of mottle-leaf. Although much work has been done with inorganic toxic agents other than lithium, the inclusion of many more organic and inorganic toxic substances in such studies may help to clarify our knowledge of the nutrition of trees under irrigated agriculture.

Summary

1. Mottle-leaf of citrus has been produced artificially in sand and soil cultures as well as in the field by the use of lithium. The lithium is assumed to act as a poison to the growth processes going on within the leaves, so that they are unable to utilize fully the inorganic salts in the tracheal sap, and as a consequence of the effect of the poison on the growth processes the old mottled leaves have the composition of immature normal leaves.

2. Analyses of the sap of comparable portions of mottled and normal citrus trees in the field have shown that, although the leaves of mottled trees may show a deficiency of calcium, the tracheal sap may contain large concentrations, possibly because of the inability of the mottled leaves to utilize it.

3. Although no lithium is at present known to occur in citrus leaves, the success in the artificial production of mottle-leaf by the use of this toxic substance adds to our understanding of the mottle-leaf phenomenon.

UNIVERSITY OF CALIFORNIA
CITRUS EXPERIMENT STATION
RIVERSIDE, CALIF.

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ORIGIN AND DEVELOPMENT OF TISSUES IN ROOT OF SCHIZAEA RUPESTRIS

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 392

DORR RAYMOND BARTOO

(WITH TWELVE FIGURES)

Introduction

The rhizome of *Schizaea rupestris* is about 2 inches in length and is densely covered with reddish brown hairs. The five or six sterile

leaves are linear, with attenuated bases and apices, and attain a length of 3 or 4 inches. The fertile leaves, borne on the same rhizome, are pinnately divided and somewhat longer stalked than the sterile ones. The sporangia are borne singly in two rows upon the abaxial side of the pinnae. Both the fertile and sterile leaves exhibit the characteristic vernation of ferns. The rhizome branches occasionally, apparently dichotomously, while leaves and adventitious roots arise from its meristematic region. The roots are quite generally branched and attain a length of about 2 inches (fig. 1).

The material upon which this study was based came from Sydney, Australia. Collections were made about November 1, when the growth of the plants was most vigorous.

The aim of this investigation was to determine the origin and development of the tissues of the root. Since all the cells of the root have a common origin in the apical cell, the study resolved itself into tracing the sequence of cell division from the immediate derivatives of the apical cell to the cells of the mature tissues. The various



FIG. 1.—Habit sketch of mature plant: *s*, sterile leaves; *f*, fertile leaves.

tracing the sequence of cell division from the immediate derivatives of the apical cell to the cells of the mature tissues. The various

structures of the root not only stand out definitely from one another, but each tissue is composed of an unusually small number of cells, enabling one to trace their lineage to the mature structures with remarkable certainty.

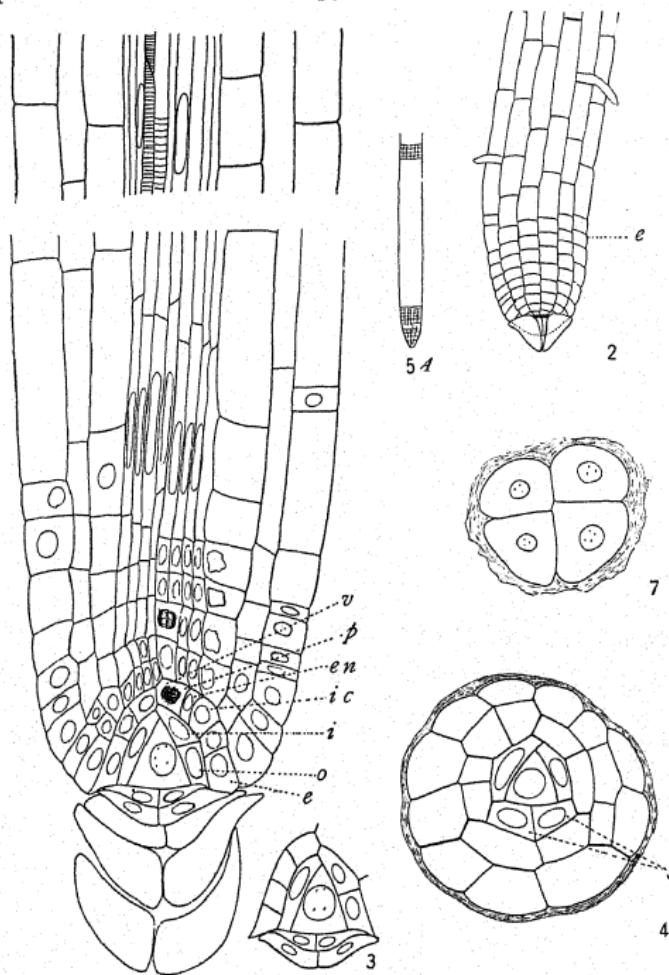
APICAL CELL AND DERIVATIVES

The root has an average length of 5 cm. and a diameter of about 0.2 mm. All the tissues arise from segments cut from a pyramidal apical cell (fig. 3); that is, the apical cell is a triangular pyramid having its longest axis in the axis of the root (figs. 3, 5). It is about one and one-half times as deep as it is broad. The segments are cut off in serial order from its four cutting faces, the derivatives from segments of three of the faces becoming tissues of the root proper, while segments cut from the fourth face form the root cap.

The lineage of the successive segments may now be traced into the distinct tissues of the mature root. As viewed in longitudinal section, each of the immediate segments divides first by a periclinal wall into an outer and an inner cell (fig. 5 *o, i*). The outer cell, by one periclinal division, gives rise to an epidermal initial (*e*), and an initial of the outer layer of the cortex (*c*). The inner cell (*i*) by two successive periclinal divisions gives rise to four cells, the outer three of which initiate the inner layer of the cortex (*ic*), the endodermis (*en*), and the pericycle (*p*) respectively. It is from the innermost of the last cells (*v*) that the desmogen strand arises (fig. 5), but the subsequent behavior of this cell depends upon its radial position. Consequently, in addition to a consideration of the sequence of cell division as it appears in longitudinal section, the cell divisions occurring in the meantime as seen in transverse section must be borne in mind.

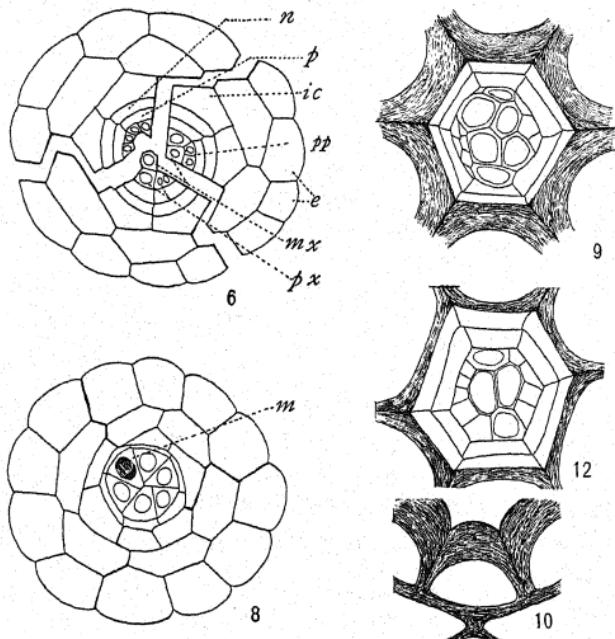
As just stated, each of the three immediate segments cut from the apical cell divides by a periclinal wall into an outer and an inner cell (fig. 5 *o, i*). Each of the three inner cells thus formed divides by a radial wall which extends from near the middle of the outer side inward, meeting the side of the segment near its inner angle. Thus is formed a sextet of cells of which the alternate three meet in the center while the remaining three do not extend to the center. This

dissimilarity of the inner sextet of cells is maintained in those subsequent derivatives which occupy similar positions at the center



FIGS. 2-5, 7.—Fig. 2, Habit sketch of root tip: *r*, root cap; *e*, epidermal cells; fig. 3, longitudinal section of apical cell; fig. 4, transverse section of apical cell; fig. 5, longitudinal section of root showing origin of tissues: *e*, epidermis; *c*, outer layer of cortex; *ic*, inner layer of cortex; *en*, endodermis; *p*, pericycle; *v*, desmogen strand initial; fig. 5a, longitudinal sketch showing relative position of two parts of fig. 5; fig. 7, transverse section of root cap near apical cell.

(fig. 8). Simultaneous with the division of the inner cell occurs the radial division of the outer cell. At this stage, then, we have a sextet of isodiametric cells inclosing a sextet of cells somewhat tetrahedral in form, as seen in transverse section. During the two successive



FIGS. 6, 8-10, 12.—Fig. 6, transverse section showing same tissue layers as fig. 5; fig. 8, transverse section of region of maturation; *m*, mitotic figure initiating pericycle and desmogen; fig. 9, transverse section through mature region: fig. 10, transverse section showing thickened wall of inner cortical cells; fig. 12, transverse section through mature region of root: *px*, protoxylem; *mx*, metaxylem; *pp*, phloem; *p*, pericycle; *n*, endodermis; *ic*, inner cortical layer.

pericinal divisions of each of the inner sextet, which immediately follows, each of the cells of the outer sextet also divides pericinally. These last pericinal divisions are followed by a radial division of each of the six cells of the outer layer, which completes the initial cells of each tissue layer (fig. 6). There are twelve cells in the epi-

dermis; six cells each in the two layers of the cortex, the endodermis, pericycle, and desmogen. No attempt was made to include, in this sequence of cell divisions, those occurring anticlinally. In general the anticlinal divisions of the epidermis, cortex, endodermis, and pericycle were several (six to ten); while probably never more than one occurs in the desmogen strand.

The subsequent behavior of each of the six desmogen strand initials depends upon its radial position. Two of the alternate three, which occupy positions at the center, divide periclinally and the two daughter cells which maintain the central position are metaxylem initials (fig. 6 *mx*), while the remaining two are destined to become phloem (*pp*). Two of the alternate three which do not occupy the central position become protoxylem initials (*px*). Not infrequently either the metaxylem initials or the protoxylem initials divide again, giving four metaxylem or four protoxylem strands. No cases were noted, however, where both the protoxylem and metaxylem initials divided in the same bundle. Each of the two remaining cells of the desmogen sextet divides once radially, and these initiate the phloem parenchyma (fig. 6); thus a diarch bundle is formed having four adjacent xylem strands, the two outer ones being protoxylem (*px*), and the two inner ones metaxylem (*mx*). It is to be noted that from each of two of the three original segments cut from the apical cell (fig. 4), both xylem and phloem arise, while the third gives rise to phloem only.

ROOT CAP

The root cap is produced by successive generations of segments cut off from the fore face of the apical cell. By two successive divisions, perpendicular to each other and also to the fore cutting face, four cells result (fig. 7). These cells grow to a considerable size but do not divide again. The cells from as many as four initial segments were observed on vigorously growing roots (fig. 5). The older cells are roughly pear-shaped and inflated on the side away from the growing point. As they become separated from the food supply their contents become less and less dense, and partial disintegration of their walls often takes place before they are sloughed off.

As is shown in both transverse and longitudinal sections, the tissues are extremely diagrammatic. The constancy of the number

of cells per layer as seen in transverse section is a striking feature; twelve for the epidermis and six each for the cortex, endodermis, and pericycle layers. While the number of phloem parenchyma strands varies from eight to twelve, the xylem almost always consists of four or six elements (figs. 9, 12). All the tissues of the root are differentiated very early. The initial cells for the epidermis, cortex, endodermis, and pericycle are only two cells removed from the apical cell, while xylem and phloem strands can be recognized soon after.

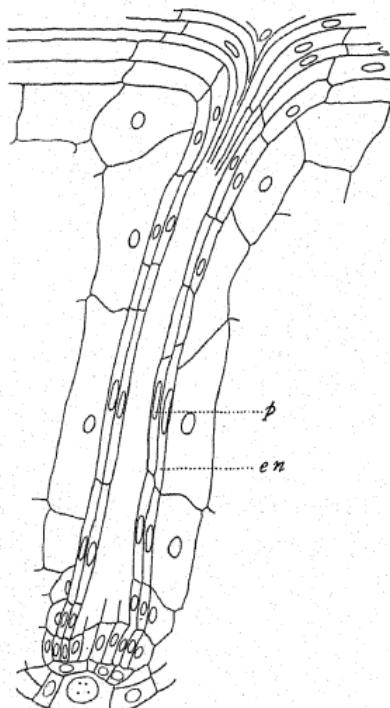
EPIDERMIS

For a distance of 0.2 mm. behind the root cap there occurs rapid cell division anticlinally (fig. 5). While most of the cells of the epidermis are isodiametric, some of them remain flattened, with their longitudinal axes much shorter than those of their neighbors (fig. 5). These smaller cells, which are destined to produce root hairs, cease to divide and can be recognized when only six cells removed from the apical cell. They do not elongate in the direction of the axis of the root, as do the other epidermal cells, but even before their sister cells have ceased to elongate their volume is increased by their extension into root hairs. Of the seventy-five root hairs observed, there were no exceptions to the fact that the epidermal cells producing root hairs do not increase to any appreciable extent except by means of extending root hairs. From differences in staining reactions it seems probable that there is a difference in density, and perhaps also a chemical difference in these cells some time before root hairs are produced. Less than one-tenth of the epidermal cells produce root hairs, even though the latter occur upon roots throughout their entire lengths.

CORTEX

The cells of the cortex keep pace in their divisions and elongation with those of the epidermis. The cell walls of the inner layer become greatly thickened upon their inner and radial faces. PRANTL (5) refers to these thick-walled cells in *Schizaea pennula* as "die sechs dickwandigen inner Rindenzellen." Miss ECKERSON found by micro-chemical tests that the corresponding thick walls in *S. pectinata* consist of cellulose with suberized and lignified lamellae (fig. 10). Thickenings on the cortical walls next to the epidermis are first evi-

dent about 0.6 mm. from the apical cell. The thickened area then spreads inward until it includes all the cells of the cortex and those of the endodermis. The thickenings to this point (0.8 mm.) are thin and uniform, but beyond this thickenings upon the inner and radial walls of the inner cortical cells continue until the lumen is reduced to at least two-thirds of its original size.



11

FIG. 11.—Longitudinal section of root rising from rhizome: *en*, endodermis; *p*, pericycle.

The pericycle cells are sister cells to those of the desmogen strand. It was noticed that their radial walls remain in the same planes with those of the endodermis and the inner cortical layer (fig. 6). The primary walls of the desmogen strand likewise remain in the same planes. This arrangement of cells might well be expected, since each tissue layer is composed of the same number of cells and since all have a common ancestor (fig. 5 *i*).

ENDODERMIS

The endodermis and inner layer of the cortex arise from a common mother cell (fig. 5). They are of equal size at first but the cortical cells soon become noticeably larger. Caspary strips were not observed, but the continuity of these cells of the fourth tissue layer (*en*) with the endodermal cells of the rhizome was easily traceable (fig. 11). PRANTL figures the corresponding endodermal layer for *S. pinnula*, while Miss ECKERSON finds that the corresponding layer in *S. pectinata* shows "caspary flecks."

PERICYCLE

PHLOEM

Adjacent to the inner side of four of the six pericycle cells arise the phloem parenchyma initials. From serial transverse sections it was impossible to distinguish sieve tube elements in the phloem parenchyma. At a distance of 0.5 mm. from the root cap the cells are uniform in size, but at a distance of 1 mm. larger ones were recognizable. It is quite probable that these larger cells are of the metaphloem, while the protophloem sieve tubes remain indistinguishable from phloem parenchyma. No sieve areas were observed in the phloem tissue.

XYLEM

As previously stated, the arrangement of the bundle is diarch, and the xylem usually consists of two protoxylem and two metaxylem cells. Each protoxylem cell lies adjacent to the metaxylem cells. At first these sister cells are similar in size and structure, but at a distance of 1 mm. from the root cap those of the metaxylem have considerably larger lumen and more rarefied contents. Wall thickenings were observed in protoxylem 0.7 mm. and in metaxylem 0.9 mm. from the apical cell (fig. 5).

In the region of elongation the desmogen strands are greatly elongated, while the cells of the cortex remain more nearly isodiametric in form. As pointed out by CHANG (3), mitotic activity ceases in the transverse plane of desmogen cells while they are still in the isodiametric form. They lengthen while their neighbors divide transversely, reaching a length of ten to twenty times their diameters (fig. 5). This is in agreement with the unpublished work of LAND, in which he states that the desmogen initials are stretched out because of the transverse divisions of the cortical cells.

Discussion

The root structure of *Schizaea rupestris* is probably the simplest and most diagrammatic of all observed roots. A transverse section of a mature root shows only three layers of cells outside the endodermis; the epidermis consisting of twelve cells and two layers of cortical cells each consisting of six cells. The single-layered endodermis and pericycle consist of six cells each. The xylem is usually of four cells (sometimes six), while the phloem varies from eight to

twelve cells. BOODLE (1) describes *S. digitale* as having twelve cells each in its epidermis and outer cortex, while the inner layer of the cortex (sclerotic cells) varies in number from the usual six to eight or nine. BRITTON and TAYLOR (2) find that the number of cells per layer corresponds in general to that of *S. digitale* and to *S. pennula* as described by PRANTL.

BOODLE finds that the radial walls of the pericycle, endodermis, and "sclerotic layer" of the cortex of *S. digitale* lie in the same radii, while the walls of the next cortical layer, which may consist of twelve cells, do not coincide with these radii. He states that the pericycle therefore appears to be cortical.

On the grounds that the endodermis and pericycle usually rise in common with the cortex, RUSSOW (6) proposed to exclude these tissues from the vascular system. STRASBURGER applied the term "phloeterma" to the inner layer of the cortex (endodermis), and considered all the tissue external to it as cortical. The recent work of CHANG on the rhizome of *Pteris aquilina* shows that the endodermis and pericycle have a common origin, and that both layers are stelar. TANSLEY and CHICK (8) state that they can hardly imagine that the sheath layers (endodermis and pericycle) are not phylogenetically identical in monostelic ferns. CONRAD (4), working on *Dicksonia punctilobula*, found that the endodermis of the root is cortical in origin, while that of the rhizome is stelar. He gives a tabulated list of thirty-four genera of ferns in which the endodermis of the roots is said to be cortical in origin.¹

PRANTL suggests that the endodermis in *Schizaea*, "just as in *Hymenophyllum*," is a part of the vascular system, for the reason that they have in many cases "without doubt" the same origin as the pericycle. He found that the endodermis and pericycle have a common origin in the smallest leaf veins of *S. pennula*.

In the present study the pericycle and endodermis are clearly of different immediate origin. The endodermis and inner layer of the cortex arise from a common initial which is sister to the cell giving rise to the pericycle and vascular elements. This is in accordance with HANSTEIN's histogenetic idea of plerome, periblem, and derma-

¹ Mostly from works of VAN TIEGHEM, DOULIOT, NAGELI, and LEITGEB.

togen. The endodermis constitutes the inner layer of the cortex and the pericycle is the outer layer of the stele.

If the distinction between stele and cortex is a morphological one, the two regions must be marked off from one another in the earliest periclinal cell divisions of the original apical cell segments. Research upon the apical cell meristem of ferns by CHANG, CONRAD, and TANSLEY (7) shows that these earliest divisions of the apical cell derivatives are inconstant; therefore this means of differentiating subsequent adult tissues is unreliable.

Although the findings of the present investigation support HANSTEIN's histogenetic idea of periblem and dermatogen, the data here presented indicate that histological distinctions are not trustworthy as a means of differentiating subsequent adult tissues of the plant.

Summary

1. All the tissues of the root have their origin in a pyramidal apical cell.
2. The root structure is probably the simplest of all observed roots.
3. Tissues of the root are differentiated extremely early; the initials of the epidermis, cortex, endodermis, and pericycle are only one cell removed from the apical cell.
4. The inner cortical layer and endodermis have a common origin; both are cortical.
5. The pericycle and desmogen strand have a common origin; both are stelar.
6. The arrangement of the bundle is diarch, consisting of four or six xylem cells and from eight to twelve phloem cells.
7. Thickening of the inner and radial walls of the "sclerotic layer" is a striking feature.
8. Epidermal cell of root hair destiny can be recognized very close to the apical region, and root hairs persist throughout the life of the root.
9. Histological distinction is unreliable as a guide to adult tissues.

The writer acknowledges with pleasure his indebtedness to Professor W. J. G. LAND, under whose direction this investigation has been conducted. He also appreciates the helpful suggestions given by Professor C. J. CHAMBERLAIN. Thanks are due to Mr. PATRICK BROUH of the University of Sydney, who made several trips to collect and fix the material used in this investigation.

TENNESSEE POLYTECHNIC INSTITUTE
COOKEVILLE, TENN.

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PROGENY RESULTING FROM SELF-POLLINATION
OF STAMINATE PLANT OF *MORUS ALBA*
SHOWING SEX REVERSAL¹

JOHN H. SCHAFFNER

In 1925, the writer (3) reported some observations on the nature of the sexual expression of ten white mulberry trees which were grown from seed obtained from a reversed branch of a staminate individual. These seeds were developed through open pollination. Of the ten trees, seven were carpellate and three staminate. Later, two of the carpellate plants showed some staminate flowers and one of the staminate plants also developed a slight amount of femaleness. The tree from which the seed was obtained showed a very interesting condition, in that one large branch developed the partial sex reversal while the other branches were typically in the secondary male state. The branch continued to produce staminate, carpellate, and mixed catkins for a number of years, until the tree was destroyed.

In the spring of 1924, a staminate tree near the botany-zoology building on the Ohio State University campus showed sex reversal in several small branches. Two of these branches were promptly covered with cloth, with some staminate branches included to insure self-pollination. A small quantity of self-pollinated seed was procured, from which fifty-five seedlings were developed in the botanical greenhouse. These little trees were transplanted out of doors in the spring of 1925, and thirty-two of them survived until the spring of 1928. In 1927, two of these trees bloomed and both were pure staminate in expression. Both of these plants bloomed again in 1928, along with four others, making a total of six in bloom. One of the plants which bloomed in 1927 was again pure staminate, while the other was prevailingly staminate but with several carpellate or partly carpellate catkins. The four new trees to come into bloom were as follows: two were pure carpellate in sexual expression; the other two were carpellate, each with a few staminate flowers.

¹ Papers from the Department of Botany, the Ohio State University, no. 226.

These six trees, therefore, derived from a selfed staminate plant, showed all the possible general sex conditions: pure female, pure male, female with reversed branches, and male with reversed branches. The potentialities which were transmitted showed that the original male condition of the parent tree possessed all the essentials for both maleness and femaleness. The development of maleness in the original staminate parent, therefore, was not due to any differential factor or chromosome condition of the cells; for maleness changed to femaleness in several vegetative branches through the ordinary somatic karyokinesis. And since the same branches also continued to produce staminate catkins or to revert back to the staminate condition, the change was not due to the loss of a chromosome or to the crossing over of a gene from one system to the other. The reduction division which segregated the chromosomes with the hereditary factors took place after sex reversal had been accomplished; and as is well known, the sex of the gametophyte was not disturbed or changed by the shifting of the heredity, but was continued in the same condition as the branch from which the gametophytes had their origin.

It is possible that no reduction took place and that the eggs developed parthenogenetically, of course; but if this were the case, it is evident that the several kinds of sex determinations were no more dependent on chromosomes or factor combinations or balances than if the reproductive processes are assumed to have developed normally.

It is now desirable that experiments be carried on with selfed carpellate plants; but however these may turn out, we know that the carpellate plants also frequently undergo sex reversal in some of their branches, giving rise to functional staminate flowers (2). Thus just as in the case of the staminate plants, the original sex condition of the carpellate individuals was not established because of the absence of a potentiality to produce a male state, nor any of the hereditary factors necessary for the development of all the normal secondary and primary male states and characters. The original female condition in such cases was plainly established in the presence of such factors. Both the original zygotes from which the staminate and carpellate plants develop have complete potentiality for

the production of maleness and femaleness. The difference in sex determination and sex expression is therefore not due to any differential in heredity, but is brought about by some difference in functional level of the two zygotes involved. This difference in functional or physiological level is brought about through many different causes, among which are to be reckoned both the external environment and the internal food condition, together with the metabolic gradient of the egg at the time of fertilization. The sex reversals which occur in the developed and differentiated organism are brought about by the same conditions in the presence of the potentialities for both the male and female states. These changes are brought about in spite of a certain sex differentiation as well as somatic differentiation, which has taken place in the entire plant, and which is often evident in the external morphology as well as in the deeper physiological processes, involving catalase and other activities.

In *Mercurialis annua*, YAMPOLSKY (9) found from limited experiments that maleness tended to perpetuate maleness and femaleness tended to perpetuate femaleness; in that his self-pollinated, reversed, staminate individuals produced male offspring and his reversed, carpellate individuals produced female offspring. *Mercurialis* evidently has a hereditary constitution of such a nature that the eggs and sperms coming from an original male soma continue in a high metabolic state which prevents the resulting zygote from falling over to the female condition, at least under the ordinary environments employed; while the eggs and sperms coming from an original female soma do not rise to the metabolic level in which the sexual state could fall to maleness.

HIRATA (1) carried on a considerable number of experiments by selfing reversed hemp plants, and also by crossing reversed plants with each other. From his experiments it is evident that a considerable percentage of the seeds did not sprout, and some, as would be expected, failed to reach maturity. A summary of his main results, including the F_1 progeny, is shown in table I.

Although less than 60 per cent of the seed planted came to maturity, the results seem to indicate that females tend to produce females while males tend to produce both males and females. The

experiments on the latter proposition are too few to give definite conclusions. The experiments do show, however, that both males and females come from either condition. The great predominance of female intersexes in experiment 3 was no doubt due to the time of planting, since the dates given run from December to January. The writer has shown that the percentage of "pure individuals" to "intersexes" is dependent on the environment in which the plants develop. One can keep the "intersexes" at zero or raise them to 90 per cent or more at will. Genetically it is of no importance whether the individual for the time being is unisexual or bisexual

TABLE I

| | SEEDS PLANTED | SEEDS SPROUTED | FEMALES | FEMALE INTERSEXES | MALES | MALE INTERSEXES |
|--|------------------|-------------------|---------|----------------------|-------|--------------------|
| 1. Female intersex selfed..... | 294 | 143 | 78 | 45 | 3 | 0 |
| 2. Mutual crossing of female intersexes..... | 48 | 24 | 12 | 4 | 1 | 0 |
| 3. Mutual crossing of female intersexes..... | 345 | 242 | 48 | 186 | 0 | 0 |
| 4. Female \times female intersex..... | 50 | 41 | 27 | 8 | 1 | 0 |
| 5. Female \times female intersex..... | 55 | 48 | 37 | 8 | 0 | 0 |
| 6. Female \times male intersex..... | 89 | 59 | 28 | 0 | 6 | 22 |
| 7. Female \times male intersex..... | 16 | 13 | 4 | 0 | 3 | 3 |
| 8. Female intersex \times male intersex..... | 38 | 25 | 13 | 0 | 9 | 0 |
| 9. Male intersex \times male intersex..... | 18 | 10 | 6 | 0 | 2 | 0 |

in expression, except that the bisexual condition gives an opportunity for selfing. Individuals that happen to be developed as pure male or pure female can be brought to the bisexual condition through proper rejuvenation treatment (7).

HIRATA's experiments plainly show that sex determined in the seed is not due to any homozygous-heterozygous shifting of chromosomes or sex heredity; for just as staminate plants can change to carpellate and carpellate to staminate after their sex has been determined, so a hereditary constitution which is determined as male can produce both male and female individuals through the zygote, and a hereditary constitution which is determined as female can in the same way produce both male and female individuals. The breeding experiments on hemp as well as the fact of the abundance of reciprocal sex reversal, which is so easily brought about under proper ecological conditions, show conclusively that no matter what the

chromosome constitution of hemp may be, the facts do not lend themselves to any plausible explanation on the basis of the hypothetical "XY type" of inheritance.

In hemp, as in the mulberry, it is no more a question of determining whether one or the other sex is homozygous or heterozygous (we know that they are both heterozygous if the word means anything), but it is a problem of great interest to discover the functional conditions of the gametes produced on the two sexes and the sex ratios of the resulting progeny.

As intimated, it is evident that in all cases of sex reversal in monosporangiate species, like hemp, Japanese hop, Jack-in-the-pulpit, tall meadow-rue, etc., the original development of the given sexual state cannot be ascribed directly to any differential hereditaries they may contain, nor to any chromosome differences, since at a later stage of the ontogeny, the very same hereditary constitution and the very same complement of chromosomes give rise to the opposite sex expression. In terms of the Mendelian hypothesis of sex, the chromosomes or the hereditary factors would thus be determining exactly the opposite from what they had done before. It must be evident that if the physiological condition of the cell or tissue can re-determine the sexual state and the sexual characters after differentiation in the original sexual state has been accomplished, there is no basis for a blind faith in the assumption that in the zygote the sex was not determined in exactly the same way, but by the special set of allosomes or sex genes present. If these were the causal agents in the zygote, why are the so-called "sex chromosomes" found to be entirely passive at the reversal stage? It must be recognized that the Mendelian sex hypothesis was an assumption in the first place, which needed proof from all unisexual species for its serious consideration. The mere coincidence, in certain cases, of definite allosome sets with definite sexual states does not prove that the two have the relation to each other of cause and effect, even if such correspondence were universal, which is very far from the facts of the case. In the higher plants the correspondence of unisexuality of the individual with a special allosome condition is comparatively very rare, and for the gametophyte there is no correspondence at all.

Studies on the resulting sexual states from selfed reversed individuals should be extensively made, for the results will lay a firm foundation of fact on which to establish a fundamental theory of sex determination. The experiments carried on so far indicate that quite different results are to be anticipated from different hereditary constitutions reacting in normal environments as well as in abnormal ones. Whatever the actual results, the interpretations must be in harmony with the conditions found in general for the species. This has not been done heretofore. It is also evident that if changing physiological states can bring about complete sex reversal in the soma, similar reversals can also be accomplished in the zygote. The only problem is to find efficient ecological factors to induce the proper functional states.

Summary

1. A reversed staminate tree of *Morus alba* was self-pollinated and produced good seed from which thirty-two young trees were grown. Of these the first six to bloom had sex expression as follows: one tree was pure staminate for two years; one was pure staminate the first year and of mixed sex the second year; two were pure carpelate; and two were carpellate with a slight development of staminate catkins.
2. Determination of the sex of the original male parent, therefore, was not due to any sex-determining differential heredity present, since it proved to have the potentialities for both sexes, as shown by the reversal in its soma; and the sex of the offspring was also determined in the zygote as male and female, through a physiological gradient which caused a male or a female state to be differentiated in the same manner as and through the same cause as that which induced these states to develop through sex reversal in the advanced ontogeny of the individual. Sex-determining factors and sex-determining chromosomes can have no correspondence with the phenomena brought out by the experiment.

OHIO STATE UNIVERSITY
COLUMBUS, OHIO

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IRREVERSIBLE INJURY AND CO₂ PRODUCTION FROM CELLS OF *NITELLA FLEXILIS*

P. A. DAVIES

Introduction

It is a well established fact that CO₂ is produced abnormally in stimulated and injured tissue. The mechanism of CO₂ production by "dead" tissue is in doubt. To say that a cell is dead because it fails to meet certain criteria may be erroneous, for the cell may be injured to such an extent that it is unable to respond to certain tests, and yet retain certain vital properties. HAAS (3, 4), using *Laminaria* tissue, found an increased rate (above the normal) of CO₂ production from dead cells, death being determined by a conductivity method (described by OSTERHOUT 8). Irreversible injury and death are not synonymous terms, for irreversible injury may be considered to occur at that point on a curve of injury beyond which the cell fails to recover beyond a certain point; while death signifies complete inability to recover to any degree.

This study is concerned primarily with the production of CO₂ from irreversibly injured cells of *Nitella flexilis*.

Material and methods

The cells of *Nitella flexilis* were rigid and separated freely from one another, so that a large number could be used with assurance of complete circulation around each cell. The number used for each experiment varied between 75 and 125 large cells, with an approximate average of 6.23 smaller rosette cells for each large cell.

The apparatus used was that evolved by OSTERHOUT (9). Before being placed in the apparatus, all material was first brought to the desired temperature (30° C.). In every experiment a normal rate of CO₂ production was established before the reagent (ethyl alcohol) was added. In order to consider the production of CO₂ by cells irreversibly injured, the irreversibility of the injury must be assured. In establishing a criterion of such injury the following methods were employed: (1) loss of rigidity of the cellular struc-

ture;¹ (2) cessation of cytoplasmic streaming; (3) inability of the cell to accumulate cresyl blue; and (4) non-recovery under optimum conditions. The writer found that in every case where the rigidity of the cell had disappeared cytoplasmic streaming had stopped,² the cell failed to accumulate cresyl blue, and no recovery took place after seven days under optimum conditions. The loss of rigidity is therefore taken as an index of irreversible injury.

Results

In working with uniform material under constant conditions, variations were found in the time of exposure to a definite concentration before the appearance of irreversible injury (loss of rigidity). These variations must be due to differences among the cells.³ Table I shows the time required for cells to lose their rigidity in different concentrations of alcohol. It will be observed that the time required for cells from the same lot of material to lose their rigidity varied greatly; for instance, in the 10 per cent solution a variation latitude of 20 minutes and 19 seconds is shown. As the concentration increases, the latitude of variation becomes less. Due to the variation of material, a variation in CO₂ production was obtained; so that each average shown (table II) represents the average of three to thirteen experiments.

When alcohol was added to the material in the apparatus (after a normal rate of CO₂ production was established), an initial rise in the rate of CO₂ production was induced. This rise is not specific for the action of alcohol on cells of *Nitella flexilis*, for such a rise has been observed by IRVING (5) when young shoots of *Hordeum vulgare* were subjected to low concentrations of chloroform; HAAS (3, 4) when

¹ The loss of rigidity of the cell was determined by means of a glass rod. One end of a glass rod 5 mm. in diameter was drawn out into a smaller diameter (1 mm.) and this was bent into a hook. If a cell bent across this hook so that when removed from the solution the free ends touched and failed to separate, the rigidity of the cell was considered lost.

² KÜHNE (6) and NOTTMANN-ZUCKERKANDL (7) found that cytoplasmic streaming stopped without recovery when the cells of *Nitella* were subjected to high concentrations of narcotics.

³ The material was kept in the laboratory in an aquarium in which the water was changed daily, the temperature of which was approximately 18° C. A new lot of the material was secured every 10-12 days.

alcohol, acetone, ethyl chloride, and formaldehyde were added to *Laminaria* tissue; GUSTAFSON (2) with formaldehyde, ether, and acetone on *Aspergillus niger*; BROOKS (1) with ether solution on *Bacillus subtilis*; and RAY (10) when *Ulva* tissue was subjected to low concentrations of chloroform.

The height of the initial rise in CO_2 production depends on the concentration of the alcohol. A 20 per cent solution caused the highest initial rise, followed by the 10, 30, 40, 60, and 95 per cent solutions respectively. In the 10 per cent solution, the alcohol did not penetrate the cell structures rapidly enough (determined by the

TABLE I

TIME REQUIRED FOR CELLS OF *NITELLA FLEXILIS* TO LOSE RIGIDITY IN DIFFERENT CONCENTRATIONS OF ETHYL ALCOHOL; TEMPERATURE 36° C.

| PERCENTAGE ETHYL ALCOHOL (BY VOLUME) | TIME REQUIRED FOR FIRST CELL TO LOSE RIGIDITY | | TIME REQUIRED FOR LAST CELL TO LOSE RIGIDITY | | AVERAGE TIME FOR LOSS OF RIGIDITY IN ALL CELLS TESTED | |
|--|--|---------|---|---------|---|---------|
| | Minutes | Seconds | Minutes | Seconds | Minutes | Seconds |
| 10..... | 25 | 4 | 45 | 23 | 33 | 58 |
| 20..... | 2 | 10 | 3 | 38 | 3 | 19 |
| 30..... | 1 | 7 | 1 | 35 | 1 | 18 |
| 40..... | | 30 | | 49 | | 39 |
| 60..... | | 15 | | 22 | | 19 |
| 95..... | | 4.5 | | 7 | | 5.9 |

time required for loss of rigidity) to cause a rapid release of CO_2 induced by the 20 per cent solution; in the 30, 40, 60, and 95 per cent solutions the alcohol caused a rapid change of the cell structure to such an extent that CO_2 was released slowly from the irreversibly injured cells, and was taken up by the solution as it escaped, according to the coefficient of CO_2 absorption by the solution.

The OSTERHOUT apparatus is so constructed that only when the CO_2 equilibrium of the system is established is the rate produced by the material accurately measured. With the sudden increase (initial rise) in the rate of CO_2 production, the CO_2 equilibrium is disturbed to such an extent that the production of CO_2 after the initial rise cannot be measured accurately until reestablishment of the equilibrium. The CO_2 measured between the disturbance and reestablishment of the equilibrium is the CO_2 produced by the initial rise plus the CO_2 normally produced after the initial rise.

If irreversible injury takes place before the reestablishment of the CO_2 equilibrium, the measured CO_2 at that time will show a higher rate than is produced by the material. The high rate would cause a delay in the drop of CO_2 production below the normal. In all concentrations, except the 10 per cent solution, irreversible injury occurred before the reestablishment of the CO_2 equilibrium.

Table II shows a comparison of the average time for loss of rigidity (irreversible injury) and the average time for CO_2 production to drop below the normal rate. It will be observed that the greatest delay (time between irreversible injury and drop of CO_2

TABLE II

COMPARISON OF AVERAGE TIME FOR LOSS OF RIGIDITY AND AVERAGE TIME FOR CO_2 PRODUCTION TO DROP BELOW NORMAL RATE AFTER ALCOHOL WAS ADDED

| PERCENTAGE ETHYL ALCOHOL (BY VOLUME) | AVERAGE TIME FOR LOSS OF RIGIDITY IN CELLS TESTED | | AVERAGE TIME FOR CO_2 PRO- DUCTION TO DROP BELOW NORMAL AFTER ALCOHOL ADDED | | DIFFERENCE BETWEEN TIME FOR LOSS OF RIGIDITY AND TIME FOR CO_2 PRODUCTION TO DROP BELOW NORMAL | |
|--|--|---------|---|---------|--|---------|
| | Minutes | Seconds | Minutes | Seconds | Minutes | Seconds |
| 10..... | 33 | 58 | 32 | 16 | 1 | 42 |
| 20..... | 3 | 19 | 8 | 30 | 5 | 11 |
| 30..... | 1 | 18 | 3 | 12 | 1 | 54 |
| 40..... | | 39 | 2 | 13 | 1 | 34 |
| 60..... | | 19 | 1 | 22 | 1 | 3 |
| 95..... | | 5.9 | | 53 | | 47.1 |

production below the normal rate) occurred in the 20 per cent solution; also, that as the concentration increased the delay became less. As already explained, the highest initial rise occurred in the 20 per cent solution; and as the concentration increased, the height of the initial rise decreased. It would appear, therefore, that the delay in the drop of CO_2 production below the normal rate must be due to the CO_2 produced by the initial rise. In the 10 per cent solution, the period between the addition of alcohol and irreversible injury was of such a magnitude that the CO_2 equilibrium was established before irreversible injury occurred, so no delay was noticeable.

The data apparently indicate that the rate of CO_2 production drops below the normal at the time of, or very shortly after, irreversible injury occurs. The findings are not in agreement with those of HAAS (3, 4), for he found the rate of CO_2 production by *Laminaria*

tissue to be higher than the normal for an extended period after the cells were dead.

Summary

1. The data would seem to indicate that the rate of CO_2 production from cells of *Nitella flexilis* drops below the normal at the time of, or very shortly after, irreversible injury occurs.
2. The findings are not in agreement with those of HAAS, who found an increased rate (above the normal) of CO_2 production by *Laminaria* tissue for an extended period after the cells were dead.

LABORATORY OF PLANT PHYSIOLOGY
UNIVERSITY OF LOUISVILLE
LOUISVILLE, KY.

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BRIEFER ARTICLES

ARTIFICIAL CULTURE OF *GANODERMA LUCIDUS* LEYSS FROM SPORE TO SPORE

(WITH ONE FIGURE)

Ganoderma lucidus is a saprophyte as well as a wound parasite. COLEMAN¹ has recently made a very thorough and interesting study of the nature of the spore wall in this species. He states that after repeated attempts he could not obtain a single germination of its spores.

For purposes of spore culture, a fresh sporophore was collected in February, 1927, from the suburb of Calcutta. It was thoroughly washed in sterilized distilled water. A petri dish containing sterilized malt extract agar medium² was taken, and on the inner surface of the upper lid a small piece of the sporophore was attached by means of melted agar in such a way that the porous surface remained pointing downward above the medium, when the upper lid was put in position. The petri dish in this condition was kept within a bell jar lined by water-soaked blotting paper to maintain the proper humidity of the inclosed air. The next day the petri dish was removed from the bell jar and examined under the low power of the microscope. It was found that a number of spores had dropped on the medium, and that some had germinated into mycelium.

Various media, such as wood decoction agar, prune agar, etc., had been tried for germination of spores of this fungus, before it was finally found that spores could be germinated in the malt extract agar medium, with a pH of 6.9. At once mycelial transfers were made aseptically by means of a platinum loop to a number of sterile tubes containing the same sterilized medium. These were kept within a glass chamber from February to August. During this period the room temperature varied from 77 to 95° F., and the relative humidity of the glass chamber varied from 62 to 74 per cent. At first growth was rather slow. In the course of about a month and a half the whole of the slants became covered with white fluffy growth, which gradually thickened, and there was exudation of a number

¹ COLEMAN, L. C., Structure of spore wall in *Ganoderma*. BOT. GAZ. 83: 48-60. 1927.

² Agar 2 per cent, malt extract 3 per cent, water 100 cc.; agar for this purpose was washed overnight in running water, then washed in distilled water and boiled.

of small glistening drops from the white hyphae and a number of crystals of calcium oxalate were found on the hyphae. No discoloring of the malt extract agar medium was noticed, nor any formation of conidia. The hyphae were closely septate and much branched, and clamp connections were quite common. Growth within the tubes declined at the end of about two months from the date of inoculation, and continuous subcultures to fresh tubes with the same medium were carried on almost every month from March until the present time. In this way the growth has been kept up. Some of these subcultures showed the formation of loose

pores without any pilear form on the white mycelial surface, within which typical mature brown spores of *Ganoderma lucidus* were found after an interval of about a week from the date of inoculation.

In April, 1927, mycelial transfer was made from one of the tubes to a sterilized block of dead but compact wood of *Mangifera indica* within a sterile Roux tube with a little sterile water at the constricted end. This tube was kept at ordinary room temperature within the same glass chamber. The mycelial growth on the block of wood was poor, the growth being confined to the upper

part of the block. The wood showed no external sign of decay. During July the white mycelial mat on the top surface of the block gradually acquired a pinkish tinge, while the outer brim showed a number of pore formations (irregular hymenial patch), the white pores being pointed upward. By the end of August, that is, after an interval of about five months, a tiny fructification in the form of a pinkish-white hemispherical hump was formed on the top of the wood block (fig. 1). It showed three concentric rings in the form of a zonation on the upper surface, which was gradually acquiring a deeper tint. The fructification resembles in form the cushion-like expansion figured and described by WHITE.³

A fragment taken aseptically from the top surface of the wood block by means of a platinum loop, and examined under the microscope on

³ WHITE, J. H., Biology of *Fomes appplanatus* (Prs.) Wallr. Trans. Royal Canadian Inst. Toronto. 1919.

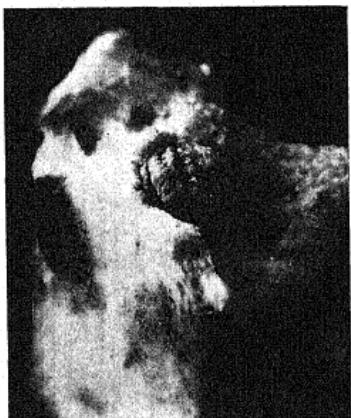


FIG. 1

August 26, showed the formation of a number of typical spores of *Ganoderma lucidus*. The color of the spore wall was not quite brown, however, not being fully mature, and showed a lighter (yellowish) shade. I could get the spores at different stages as figured by COLEMAN in the article already referred to. Soon after, the deposit of spores on the sides of the Roux tube could be distinctly seen in the form of a cloudy mass.

Mycelial bits from a pure culture were also transferred to sterilized wood blocks of *Spondias mangifera* and *Tamarindus indica* in August, 1927. One of them, that on *Spondias mangifera*, has given rise to a number of typical spores, although no regular sporophore has been formed as yet. Both of these wood blocks show poor mycelial growth on their upper part. They are exuding copiously glistening drops of liquid.

It is my experience with the artificial cultures of a number of fleshy fungi that the fruiting bodies hardly assume the normal form. This might be due to the artificial conditions of restricted air supply, light, and moisture, under which they grow.

Incidentally, while studying the cytology of the basidia of some sporophores of this species, I was able to demonstrate by GOLGI's bichromate and silver-nitrate method a fine blackened net within the basidium, which recalls the nature of the GOLGI apparatus in animal cells.—S. R. BOSE, Carmichael Medical College, Calcutta, India.

[Accepted for publication March 2, 1928; delayed through lost correspondence]

CURRENT LITERATURE

BOOK REVIEWS

Silviculture

In a recent volume TOUMEY¹ has shown the close harmony existing between silviculture and plant ecology. Ecologists are accustomed to say that all forestry is but applied ecology, but TOUMEY claims that "the foundation of silviculture as we conceive it today, is not an outgrowth of plant ecology, but rather plant ecology is an outgrowth of it," and it is doubtless true that silviculture was well established before ecology was recognized as a separate branch of biological science. It is also true that such work as that of EBERMAYER, begun in 1861, in forestry experiment stations, was good plant ecology of the truly scientific and experimental type. To these stations is credited the beginnings of the era of modern scientific silviculture.

TOUMEY credits plant ecology with many contributions to silviculture, the most important of which is the development of the concept that the plant community is a fundamental unit of vegetation. On the other hand, he says that plant ecologists have seized upon the methods of the silviculturist in the study and measurement of habitat or site factors. This seems an ideal cooperation, and this unity of aim and similarity of purpose in ecologist and silviculturist permeates the entire volume; in fact, ecologists would do well to study the book carefully and might profit by adopting it as one of their textbooks.

The author proposes to divide the discussion of his subject into three parts: site factors, forest vegetation, and methods of investigating site factors and forest vegetation and relating the one to the other. Only the first and second parts are included in the present volume; the discussion of the third will appear later.

The scope of part I may be gathered from such chapter headings as definitions and generalities; climatic factors; physiographic factors, and biotic factors; and reaction of forest vegetation on the site factors. The discussion of part II is under such heads as forest vegetational units and their classification; life history of forest communities; the stand; the tree.

While all topics are well discussed, particular mention might be made of the careful consideration of light and the definition and analysis of "tolerance." Excellent diagrams help to illustrate and to separate the combined effect of light and soil moisture on reproduction and undergrowth. Among the physiographic factors, forest humus and soil moisture receive very careful attention. In connection with the latter, TOUMEY's own contribution on the characteristic forms of initial root habit and their relation to available soil moisture is very valuable

¹ TOUMEY, J. W., *Foundations of silviculture upon an ecological basis*. Vol. I. 8vo. pp. 438. *figs. 11.* New York: John Wiley & Sons. 1928.

and noteworthy. Biotic factors are more briefly discussed, mycorrhizas in particular being dismissed with rather scant attention.

In the classification of forest communities, the familiar ecological systems of SCHIMPER and WARMING are sketched; and for North America those of SARGENT, CLEMENTS, SHREVE, and ZON are outlined in more detail. In the matter of forest types, the discussion becomes more strictly that of the forester, although much stress is placed on CAJANDER'S system of basing the classification on indicator plants found in the undergrowth of the forest. While this system is not without its difficulties, it seems about the best available to evaluate the potentialities of the site. In the life history of forest communities, the principle of succession is recognized and applied to problems of establishment and reproduction.

Throughout the entire volume the basic fundamental principles are sought and the discussion is clear and logical. The organization is excellent and the bibliography shows a thorough familiarity with ecological as well as with forestry literature. Its viewpoint and scope make it useful and instructive to the ecologist, and indispensable to the forester.

A second book on silviculture by HAWLEY² is from the same laboratory and by the same publishers, but presents a very different viewpoint. It deals with the practical application of the principles discussed by TOUMEY. Its aim is to direct the forester how to produce and manage a forest in such a manner as shall best fulfill the objects of the owner. It seems to discuss its problems with clearness and vigor, and to bring to the discussion the best technical knowledge available.

The contents of the volume may be judged by such chapter headings as: reproduction methods; clear cutting methods; seed tree method; shelter wood method; selection method; coppice method; intermediate cuttings; slash disposal; forest protection; fire control; protection against insects; and protection against tree diseases. Its organization is such that the topics follow one another in orderly succession, and it is well illustrated with diagrams and charts. An extensive bibliography is cited, the citations being arranged in lists at the end of each chapter, while an extensive index seems to make all topics readily accessible.

Both books are well printed and bound, and will form valuable additions to ecological and forestry classics.—G. D. FULLER.

Colloid chemistry

A remarkable collection of papers has been brought together in the second volume on colloid chemistry, edited by ALEXANDER.³ This volume contains the

² HAWLEY, R. C., *The practice of silviculture with particular reference to its application in the United States of America*. 8vo. 2d.ed. pp. 335. figs. 69. New York: John Wiley & Sons. 1929. \$4.

³ ALEXANDER, JEROME, *Colloid chemistry*. Vol. II. 8vo. pp. 1029. Chemical Catalog Co., New York. 1928.

papers in the field of biology and medicine; the first volume was issued some time ago, and covered the general theoretical field. The third volume will consider industrial applications of colloidal chemistry and physics.

The book contains fifty-seven monographs on many different phases of colloidal behavior. Many of the authors have won international fame for their work in special phases of colloidal action, and it is an extremely valuable addition to the literature of colloids, mainly because of the character of the authorship. It is not possible in a brief review to give an adequate idea of the value of the various monographs. However, the names of some of the authors and titles of papers are mentioned to show the unusual character of the book. STÉPHANE LEDUC contributes a paper on solutions and life; Sir WILLIAM BRAGG discusses colloids and X-rays; WOLFGANG PAULI considers proteins as colloids; G. BREDIG has a paper on inorganic ferments; RICHARD WILLSTÄTTER writes on adsorption of enzymes; E. B. WILSON discusses physical basis of life; F. D'HERELLE presents an account of the bacteriophage, a living colloidal micell. D. T. MACDOUGAL considers arrangement and action of the colloids of the plant cell; RUDOLPH HÖBER has an account of colloidal state and physiological function; papers by BOTTAZZI, FISCHER, SEIFRIZ, HEILBRUNN, R. S. LILLIE, CHAMBERS, and LUMIÈRE deal with protoplasm as a system of colloids. There are papers on the colloidal features of tuberculosis, malignant tumor, acute inflammation, lobar pneumonia, and cancer.

The appendix reprints a paper by JACQUES LOEB, an explanation of the colloidal behavior of proteins, which was the PASTEUR Lecture delivered before the Institute of Medicine of Chicago, November, 1922. This was in lieu of a special paper which LOEB's untimely death kept him from writing.

The reviewer considers it one of the best collections of papers in this field. The size of the volume makes it expensive, but in these days of high book prices one can easily spend more and obtain less for his money. Libraries should not be without it, if colloidal literature is given a place on the shelves.—C. A. SHULL.

Statistical methods for research workers

The second edition of FISHER's⁴ work on statistical methods, appearing so soon after the first edition (1925), is proof of the popularity of this work, and evidence of the great need for such a monograph for students of biological problems. The problem of variability is always present in any biological analysis, and every man sooner or later finds that he must employ the methods of statistics in testing the accuracy of his results and the validity of his conclusions.

FISHER has tried to avoid the difficulties of the mathematical theory of statistical methods, and aims to present the matter from the standpoint of practical procedure, appropriate to the problems in hand. This permits omission of mathematical proofs, which unfortunately few biologists are well enough trained in mathematics to follow, and yet gives ample opportunity to present

⁴ FISHER, R. A., *Statistical methods for research workers*. 8vo. pp. xi+269. Oliver and Boyd, Edinburgh and London. 1928.

the practical applications of statistics to biological data. FISHER's wide experience as chief statistician at Rothamsted has brought him into contact with the needs of the man who must cope with small sample problems. He gives us the advantage of his experience in this monograph.

There are nine chapters, the last of which, on principles of statistical estimation, is new in this edition. After an introductory chapter, which considers the scope, methods of calculation, and qualifications of satisfactory statistics, he takes up diagrams; distributions; tests of goodness of fit, independence, and homogeneity; tests of significance of means, differences of means, and regression coefficients; the correlation coefficient; intraclass correlations and the analysis of variance; and further applications of the analysis of variance. The additional ninth chapter takes up the significance of the evidence for linkage, specification of the progeny population for linked factors, the multiplicity of consistent statistics, comparison of statistics by goodness of fit tests, sampling variance of statistics, and the interpretation of the discrepancy x^2 .

A number of the tables most used in actual work are repeated at the end of the book, so that they can be detached and mounted for convenience of reference. These include the table of x , table of x^2 , table of t , table of values of the correlation coefficient for different levels of significance, table of r for values of z from 0-3, and tables of the 5 and 1 per cent points of the distribution of z .

The work is not intended to be used as a textbook on statistics, but as a monograph for the research student, to be mastered as a part of his necessary equipment for effective research. Students who lack sufficient mathematics to handle FISHER's monograph should consider themselves deficient in that fundamental branch of science, and give some attention to algebra and analytics. The book is worthy of careful study by all who deal with problems in which variable data are collected and interpreted.—C. A. SHULL.

NOTES FOR STUDENTS

Taxonomic notes.—HUBBARD⁵ has monographed the Australian genus *Astrebla*, which is renowned in that country for its drought resistance and fodder value. It is regarded as one of the "great national assets." Four species are described, one of which is new.

POE⁶ has published a revision of the *Plantago patagonica* group, which ASA GRAY first presented as containing one species and 4 varieties. Later studies added to the species, and now POE recognizes 6 species and 9 varieties, the latter being new names or combinations.

SANDWITH⁷ has described 14 new species from British Guiana, which have been found in the collections of the last few years. They represent 11 genera and

⁵ HUBBARD, C. E., The genus *Astrebla* or Mitchell grasses. Bull. Misc. Inf. Roy. Bot. Gard. Kew, no. 7. 257-266. 1928.

⁶ POE, IONE, A revision of the *Plantago patagonica* group of the United States and Canada. Bull. Torr. Bot. Club 55:406-420. 1928.

⁷ SANDWITH, N. Y., New species from British Guiana. Kew Bull. no. 9. pp. 365-379. 1928.

9 families. Attention is especially called to "the discovery of 2 new species of the interesting little genus *Heterostemon*" (Caesalpiniaceae).

In continuation of their descriptions of new plants from tropical Africa, HUTCHINSON and DALZIEL⁸ have described 4 new species of *Dichopetalum* (Chailletiaceae), and 8 new species in 5 genera of Caesalpiniaceae.

BARTRAM⁹ has published descriptions of the mosses collected by STANLEY in Costa Rica, which he visited in the early part of 1924 and the winter of 1925-26 to investigate the flowering plants. He enumerates 272 species and varieties, representing 40 families, over one-third of which are new to Costa Rica. He also describes 42 new species and varieties, and a new genus (*Neophypnella*) belonging to the Hookeriaceae. This indicates the wealth of moss material in a region of Central America where mosses have not been collected. It is stated that "the wet mountain forests of Central America are rich in mosses, especially in epiphytic forms."

FERNALD¹⁰ has published an account of the species of *Oxytropis* occurring in the northeastern section of North America. It is so prominently a genus of western North America that he remarks that "its occurrence in the northeastern section of North America has been looked upon as exceptional." He describes and discusses 10 species, which include one new species and 2 new combinations.
—J. M. C.

⁸ HUTCHINSON, J., and DALZIEL, J. M., Tropical African plants. V. Kew Bull. no. 9. pp. 380-382. 1928.

⁹ BARTRAM, E. B., Costa Rican mosses collected by PAUL C. STANLEY in 1924-1926. Contrib. U. S. Nat. Herb. 26: Part 3. 51-114. 1928.

¹⁰ FERNALD, M. L., The genus *Oxytropis* in northeastern America. Contrib. Gray Herb. LXXXII. Reprinted from Rhodora 30:137-188. 1928.

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